nitrile (1 in 2). When the procedure is run with 5 μL of this solution under the above operating conditions, leucomycin A₅ and josamycin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 5 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of leucomycin A₅ is not more than 1.0%.

**Purity (1)**

Clarity and color of solution—Dissolve 1.0 g of Kitasamycin Tartrate in 10 mL of water: the solution is clear and colorless or light yellow.

(2) Heavy metals <1.0 ppm—Proceed with 1.0 g of Kitasamycin Tartrate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

**Water <2.48%** Not more than 3.0% (0.1 g, volumetric titration, direct titration).

**Assay**

Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (i) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Leucomycin A₅ RS, equivalent to about 30 mg (potency), dissolve in 10 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C, and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Kitasamycin Tartrate, equivalent to about 30 mg (potency), and dissolve in water to make exactly 100 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

**Containers and storage**

Containers—Tight containers.

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**Labetalol Hydrochloride**

ラベタロール塩酸塩

![Labetalol Hydrochloride](image)

C₁₉H₂₄N₂O₃·HCl: 364.87

2-Hydroxy-5-{[(1RS)-1-hydroxy-2-[(1RS)-1-methyl-3-phenylpropylamino]ethyl} benzamide monohydrochloride

2-Hydroxy-5-{[(1RS)-1-hydroxy-2-[(1SR)-1-methyl-3-phenylpropylamino]ethyl} benzamide monohydrochloride [32780-64-6]

**Description**

Labetalol Hydrochloride occurs as a white crystalline powder.

It is freely soluble in methanol, and sparingly soluble in water and in ethanol (99.5).

It dissolves in 0.05 mol/L sulfuric acid TS. Melting point: about 181°C (with decomposition).

**Identification (1)**

Determine the absorption spectrum of a solution of Labetalol Hydrochloride in 0.05 mol/L sulfuric acid TS (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Labetalol Hydrochloride as directed in the potassium chloride disc method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Labetalol Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

**pH <2.34** The pH of a solution prepared by dissolving 0.5 g of Labetalol Hydrochloride in 50 mL of water is between 4.0 and 5.0.

**Purity (1)**

Heavy metals <1.07—Proceed with 1.0 g of Labetalol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.8 g of Labetalol Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add
methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 2-propanol, water, and ammonia solution (28) (25:15:8:2) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 30 minutes: the spots other than the principal spot from the sample solution do not exceed 2 in number and are not more intense than the spot obtained from the standard solution.

Loss on drying <2.44> Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Isomer ratio Dissolve 5 mg of Labetalol Hydrochloride in 0.7 mL of a solution of n-butylboronic acid in anhydrous pyridine (3 in 250), allow to stand for 20 minutes, and use this solution as the sample solution. Perform the test with 2 μL of the sample solution as directed under Gas Chromatography. Not more than 0.1

Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A fused silica column 0.53 mm in inside diameter and 25 m in length, coated inside with methyl silicone polymer for gas chromatography in 5 μm thickness.
Column temperature: A constant temperature of about 290°C.
Injection port temperature: A constant temperature of about 350°C.
Detector temperature: A constant temperature of about 350°C.
Carrier gas: Helium.
Flow rate: Adjust the flow rate so that the retention time of the peak showing earlier elution of the two peaks of labetalol is about 9 minutes.

System suitability—
System performance: Proceed with 2 μL of the sample solution under the above conditions: the resolution between the two labetalol peaks is not less than 1.5.

System repeatability: Repeat the test 6 times under the above conditions with 2 μL of the sample solution: the relative standard deviation of the ratio of the peak area of labetalol with the shorter retention time to that of the longer retention time is not more than 2.0%.

Assay Weigh accurately about 0.3 g of Labetalol Hydrochloride, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 36.49 mg of C_{19}H_{24}N_{2}O_{3}·HCl

Containers and storage Containers—Tight containers.

Labetalol Hydrochloride Tablets

Labetalol Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of labetalol hydrochloride (C_{19}H_{24}N_{2}O_{3}·HCl: 364.87).

Method of preparation Prepare as directed under Tablets, with Labetalol Hydrochloride.

Identification (1) To a quantity of powdered Labetalol Hydrochloride Tablets equivalent to 5 mg of Labetalol Hydrochloride according to the labeled amount, add 100 mL of 0.05 mol/L sulfuric acid TS, shake, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>:

Maximum between 300 nm and 304 nm.

(2) To a quantity of powdered Labetalol Hydrochloride Tablets equivalent to 0.25 g of Labetalol Hydrochloride according to the labeled amount, add 25 mL of methanol, shake vigorously for 30 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of labetalol hydrochloride in 1 mL of methanol, and use this solution as the standard solution. Perform the test using these solutions as directed under Thin-layer Chromatography. Spot 5 μL of each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 2-propanol, water, and ammonia solution (28) (25:15:8:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot obtained from the standard solution show the same Rf value.

Uniformity of dosage units <6.10> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Labetalol Hydrochloride Tablets add 5 mL of 0.5 mol/L sulfuric acid TS and 30 mL of water, shake vigorously for 30 minutes, add water to make exactly 50 mL, and filter. Discard the first 5 mL of the filtrate, pipet 4 mL of the subsequent filtrate, add 0.05 mol/L sulfuric acid TS to make exactly V mL so that each mL contains about 40 μg of labetalol hydrochloride (C_{19}H_{24}N_{2}O_{3}·HCl), and use this solution as the sample solution. Separately, weigh accurately about 20 mg of labetalol hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.05 mol/L sulfuric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, add 0.05 mol/L sulfuric acid TS to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_{1} and A_{2}, of the sample solution and standard solution at 302 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of labetalol hydrochloride (C_{19}H_{24}N_{2}O_{3}·HCl) = M_{S} \times A_{1}/A_{2} \times V/40

M_{S}: Amount (mg) of labetalol hydrochloride for assay

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900
Lactic Acid

乳酸

\[
\text{C}_3\text{H}_6\text{O}_3 : \text{90.08} \text{ (2RS)-2-Hydroxypropanoic acid [50-21-5]}
\]

Lactic Acid is a mixture of lactic acid and lactic anhydride.

It contains not less than 85.0% and not more than 92.0% of \( \text{C}_3\text{H}_6\text{O}_3 \).

Description Lactic Acid occurs as a clear, colorless or light yellow, viscous liquid. It is odorless or has a faint, unpleasant odor.

It is miscible with water, with ethanol (95) and with diethyl ether.

It is hygroscopic.

Specific gravity \( d^20_{20} \): about 1.20

Identification A solution of Lactic Acid (1 in 50) changes blue litmus paper to red and responds to the Qualitative Tests \( 1.09 \) for lactate.

Purity

(1) Chloride \( \text{1.03} \)—Perform the test with 1.0 g of Lactic Acid. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Sulfate \( \text{1.14} \)—Perform the test with 2.0 g of Lactic Acid. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

(3) Heavy metals \( \text{1.07} \)—To 2.0 g of Lactic Acid add 10 mL of water and 1 drop of phenolphthalein TS, and add ammonium TS dropwise until a pale red color appears. Add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution from 2.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and dilute with water to 50 mL (not more than 10 ppm).

(4) Iron \( \text{1.10} \)—Prepare the test solution with 4.0 g of Lactic Acid according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 5 ppm).

(5) Sugars—To 1.0 g of Lactic Acid add 10 mL of water, and neutralize with sodium hydroxide TS. Boil the mixture with 10 mL of Fehling’s TS for 5 minutes: no red precipitate is produced.

(6) Citric, oxalic, phosphoric and \( \lambda \)-tartaric acid—To 1.0 g of Lactic Acid add 1.0 mL of water, followed by 40 mL of calcium hydroxide TS. Boil the mixture for 2 minutes: no change occurs.

(7) Glycerin or mannnitol—Shake 10 mL of Lactic Acid with 12 mL of diethyl ether: no turbidity is produced.

(8) Volatile fatty acids—Warm Lactic Acid: it does not produce any acetic acid-like or butyric acid-like odor.

(9) Cyanide—Transfer 1.0 g of Lactic Acid to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, add dropwise a solution of sodium hydroxide (1 in 10) by shaking until a pale red color develops, add 1.5 mL of a solution of sodium hydroxide (1 in 10) and water to make 20 mL, and heat in a water bath for 10 minutes. Cool, add

mL of water as the dissolution medium, the dissolution rate in 30 minutes of Labetalol Hydrochloride Tablets is not less than 75%.

Start the test with 1 tablet of Labetalol Hydrochloride Tablets, withdraw not less than 20 mL of the medium at specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 \( \mu \)m. Discard the first 10 mL of the filtrate, pipet \( V \) mL of the subsequent filtrate, and add water to make exactly \( V' \) mL so that each mL contains about 50 \mu\text{g} of labetalol hydrochloride (\( \text{C}_{19}\text{H}_{24}\text{N}_{2}\text{O}_{3}\cdot\text{HCl} \)) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of labetalol hydrochloride for assay, previously dried at 105 °C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry \( \text{2.24} \), and determine the absorbances, \( A_T \) and \( A_S \), at 302 nm.

Dissolution rate (%) with respect to the labeled amount of labetalol hydrochloride (\( \text{C}_{19}\text{H}_{24}\text{N}_{2}\text{O}_{3}\cdot\text{HCl} \)) in 1 tablet

\[
M_S = \frac{A_T}{A_S} \times V' \times V \times 1/C \times 90
\]

\( M_S \): Amount (mg) of labetalol hydrochloride for assay

C: Labeled amount (mg) of labetalol hydrochloride (\( \text{C}_{19}\text{H}_{24}\text{N}_{2}\text{O}_{3}\cdot\text{HCl} \)) in 1 tablet

Containers and storage Containers—Tight containers.
dropwise dilute acetic acid until a red color of the solution disappears, add 1 drop of dilute acetic acid, add 10 mL of phosphate buffer solution, pH 6.8, and 0.25 mL of sodium toluensulfonchloramide TS, stopper immediately, mix gently, and allow to stand for 5 minutes. To the solution add 15 mL of pyridine-pyrazolone TS and water to make 50 mL, and allow to stand at 25°C for 30 minutes: the solution has no more color than the following control solution.

Control solution: Pipet 1.0 mL of Standard Cyanide Solution, and add water to make exactly 20 mL. Transfer 1.0 mL of this solution to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, and then proceed as described above.

(10) Readily carbonizable substances—Superimpose slowly 5 mL of Lactic Acid, previously kept at 15°C, upon 5 mL of sulfuric acid for readily carbonizable substances, previously kept at 15°C, and allow to stand at 15°C for 15 minutes: no dark color develops at the zone of contact.

Residue on ignition  &lt;2.44% Not more than 0.1% (1 g).

Assay Weigh accurately about 3 g of Lactic Acid, transfer in a conical flask, add accurately measured 40 mL of 1 mol/L sodium hydroxide VS, invert a watch glass over the flask, and heat on a water bath for 10 minutes. Titrate &lt;2.50% the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS immediately (indicator: 2 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS = 90.08 mg of C₃H₆O₃

Containers and storage Containers—Tight containers.

L-Lactic Acid

L-乳酸

H₂C—OH

C₃H₆O₃: 90.08
(2S)-2-Hydroxypropanoic acid [79-33-4]

L-Lactic Acid is a mixture of L-lactic acid and L-lactic anhydride.

It contains not less than 85.0% and not more than 92.0% of C₃H₆O₃.

Description L-Lactic Acid occurs as a clear, colorless or light yellow, viscous liquid. It is odorless or has a faint, no unpleasant odor.

It is miscible with water, with ethanol (99.5) and with diethyl ether.

It is hygroscopic.

Specific gravity d₂₀: about 1.20

Identification A solution of L-Lactic Acid (1 in 50) changes the color of blue litmus paper to red, and responds to the Qualitative Tests &lt;1.00% for lactate.

Optical rotation &lt;2.49 [α]₂₀: -46° to -52° Weigh accurately an amount of L-Lactic Acid, equivalent to about 2 g of L-lactic acid (C₃H₆O₃), add exactly 25 mL of 1 mol/L sodium hydroxide VS, cover with a watch glass, and heat on a water bath for 15 minutes. Cool, and adjust to pH 7.0 with 1 mol/L hydrochloric acid VS. Dissolve 5.0 g of hexammonium heptamolybdate tetrahydrate in this solution, add water to make exactly 50 mL, and determine the optical rotation using a 100-mm cell.

Purity (1) Chloride &lt;1.03%—Perform the test with 1.0 g of L-Lactic Acid. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Sulfate &lt;1.14%—Perform the test with 2.0 g of L-Lactic Acid. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.01%).

(3) Heavy metal &lt;1.07%—To 2.0 g of L-Lactic Acid add 10 mL of water and 1 drop of phenolphthalein TS, and add ammonia TS dropwise until a pale red color appears. Add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution from 2.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and dilute with water to 50 mL (not more than 10 ppm).

(4) Iron &lt;1.10%—Prepare the test solution with 4.0 g of L-Lactic Acid according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 5 ppm).

(5) Sugars—To 1.0 g of L-Lactic Acid add 10 mL of water, and neutralize with sodium hydroxide TS. Boil the mixture with 10 mL of Fehling’s TS for 5 minutes: no red precipitate is produced.

(6) Citric, oxalic, phosphoric and l-tartaric acid—To 1.0 g of L-Lactic Acid add 1.0 mL of water, followed by 40 mL of calcium hydroxide TS. Boil the mixture for 2 minutes: no change occurs.

(7) Glycerin or mannitol—Shake 10 mL of L-Lactic Acid with 12 mL of diethyl ether: no turbidity is produced.

(8) Volatile fatty acids—Warm L-Lactic Acid: it does not produce any acetic acid-like or butyric acid-like odor.

(9) Cyanide—Transfer 1.0 g of L-Lactic Acid to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, add dropwise a solution of sodium hydroxide (1 in 10) while shaking until a pale red color develops, then add 1.5 mL of a solution of sodium hydroxide (1 in 10) and water to make 20 mL, and heat in a water bath for 10 minutes. After cooling, add dropwise dilute acetic acid until a red color of the solution disappears, add 1 drop of dilute acetic acid, and 10 mL of phosphate buffer solution, pH 6.8, and 0.25 mL of sodium toluensulfonchloramide TS, stopper immediately, mix gently, and allow to stand for 5 minutes. To the solution add 15 mL of pyridine-pyrazolone TS and water to make 50 mL, and allow to stand at 25°C for 30 minutes: the solution has no more color than the following control solution.

Control solution: Pipet 1.0 mL of Standard Cyanide Solution, and add water to make 20 mL. Transfer 1.0 mL of this solution to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, and then proceed as described above.

(10) Readily carbonizable substances—Superimpose slowly 5 mL of L-Lactic Acid, previously kept at 15°C, upon 5 mL of sulfuric acid for readily carbonizable substances, previously kept at 15°C, and allow to stand at 15°C for 15 minutes: no dark color develops at the zone of contact.

Residue on ignition &lt;2.44% Not more than 0.1% (1 g).

Assay Weigh accurately about 3 g of L-Lactic Acid, transfer in a conical flask, add accurately measured 40 mL of 1 mol/L hydrochloric acid VS.
mol/L sodium hydroxide VS, invert a watch glass over the flask, and heat on a water bath for 10 minutes. Titrate 0.5 mol/L sulfuric acid VS immediately (indicator: 2 drops of phenolphthalein TS). Perform a blank determination. Each mL of 1 mol/L sodium hydroxide VS = 90.08 mg of C₆H₁₂O₇

Containers and storage Containers—Tight containers.

Anhydrous Lactose

無水乳糖

\[ C_{12}H_{22}O_{11} \text{ (Anhydrous Lactose)} \]

Identification Determine the infrared absorption spectrum of Anhydrous Lactose, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum or the spectrum of Anhydrous Lactose RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation \( \leq 2.49 \) \( [\alpha]_D^0 \): +54.4° to +55.9°. Weigh accurately about 10 g of Anhydrous Lactose, calculated on the anhydrous basis, dissolve in 80 mL of water warmed to 50°C, and add 0.2 mL of ammonia TS after cooling. After standing for 30 minutes, add water to make exactly 100 mL, and determine the optical rotation of this solution in a 100-mm cell.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Anhydrous Lactose in 10 mL of hot water: the solution is clear, and colorless or nearly colorless. Determine the absorbance at 400 nm of this solution as directed under Ultraviolet-visible Spectrophotometry 2.24\( \alpha \), using water as the control solution: not more than 0.04.

(2) Acidity or alkalinity—Dissolve 6 g of Anhydrous Lactose by heating in 25 mL of freshly boiled and cooled water, and after cooling, add 0.3 mL of phenolphthalein TS: the solution is colorless, and not more than 0.4 mL of 0.1 mol/L sodium hydroxide VS is required to produce a pale red color or red color.

* (3) Heavy metals \( \leq 0.07 \) at between 270 nm and 300 nm.

(4) Proteins and light absorbing substances—Dissolve 1.0 g of Anhydrous Lactose in water to make 100 mL, and use this solution as the sample solution. Determine the absorbances as directed under Ultraviolet-visible Spectrophotometry 2.24\( \alpha \), using water as the control solution: not more than 0.25 at between 210 nm and 220 nm, and not more than 0.07 at between 270 nm and 300 nm.

Loss on drying \( \leq 2.41 \) Not more than 0.5% (1 g, 80°C, 2 hours).

Water \( \leq 2.48 \) Not more than 1.0% (1 g, volumetric titration, direct titration. Use a mixture of methanol for Karl Fischer method and formamide for Karl Fischer method (2:1) instead of methanol for Karl Fischer method).

Residue on ignition \( \leq 2.44 \) Not more than 0.1% (1 g).

*Microbial limit \( \leq 0.05 \) The acceptance criteria of TAMC and TYMC are 10² CFU/g and 5 × 10² CFU/g, respectively. Salmonella and Escherichia coli are not observed.

Isomer ratio Place 1 mg of Anhydrous Lactose in a 5-mL screw capped reaction vial for gas chromatography, add 0.45 mL of dimethylsulfoxide, stopper, and shake well. Add 1.8 mL of a mixture of pyridine and trimethylsilylimidazole (2:1), seal the vial tightly with a screw cap, and mix gently. Allow to stand for 20 minutes, and use this solution as the sample solution. Perform the test with 2 μL of the sample solution as directed under Gas Chromatography 2.02 according to the following conditions. Determine the peak areas of α-lactose and β-lactose, \( A_\alpha \) and \( A_\beta \), and calculate the contents (%) of α-lactose and β-lactose in Anhydrous Lactose by the following equations.

\[ \text{Content (\% of \( \alpha \)-lactose)} = \frac{A_\alpha}{(A_\alpha + A_\beta)} \times 100\text{\%} \]

\[ \text{Content (\% of \( \beta \)-lactose)} = \frac{A_\beta}{(A_\alpha + A_\beta)} \times 100\text{\%} \]

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Injection port temperature: A constant temperature of about 275°C.

Detector temperature: A constant temperature of about 275°C.

Column: A glass column 4 mm in inside diameter and 90 cm in length, packed with siliceous earth for gas chromatography coated at the ratio of 3% with 25% phenyl-25% cyanopropyl-methylsilicone polymer for gas chromatography.

Column temperature: A constant temperature of about 215°C.
Carrier gas: Helium.
Flow rate: A constant flow rate of about 40 mL per minute.
*System suitability—*
System performance: Prepare a solution with 1 mg of a mixture of α-lactose and β-lactose (1:1) in the same manner as for preparing the sample solution, and proceed with 2 μL of this solution under the above operating conditions, and determine the retention times of the peaks of α-lactose and β-lactose; the relative retention time of α-lactose with respect to that of β-lactose is about 0.7 with the resolution between these peaks being not less than 3.0.

**Containers and storage**  Containers—Well-closed containers.

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**Lactose Hydrate**

**Lactose**

乳糖水和物

\[C_{12}H_{22}O_{11}·H_2O: 360.31\]

β-D-Galactopyranosyl-(1→4)-α-D-glucopyranose monohydrate

[64044-51-5, Mixture of α- and β-lactose monohydrate]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (* *).

Lactose Hydrate is the monohydrate of β-D-galactopyranosyl-(1→4)-α-D-glucopyranose.

*It is a disaccharide obtained from milk, consist of one unit of glucose and one unit of galactose.*

*The label states the effect where it is the granulated powder.*

*Description*  Lactose Hydrate occurs as white, crystals, powder or granulated powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5%).

**Identification**  Determine the infrared absorption spectrum of Lactose Hydrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with *the Reference Spectrum or* the spectrum of Lactose Hydrate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> \([\alpha]_D^{20} + 54.4° - +55.9°\). Weigh accurately about 10 g of Lactose Hydrate, calculated on the anhydrous basis, dissolve in 80 mL of water warmed to 50°C, and add 0.2 mL of ammonia TS after cooling. After standing for 30 minutes, add water to make exactly 100 mL, and determine the optical rotation of this solution in a 100-mm cell.

**Purity (1)**  Clarity and color of solution—Dissolve 1.0 g of Lactose Hydrate in 10 mL of hot water: the solution is clear, and colorless or nearly colorless. Determine the absorbance at 400 nm of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control solution: not more than 0.04.

(2)  Acidity or alkalinity—Dissolve 6 g of Lactose Hydrate by heating in 25 mL of freshly boiled and cooled water, and after cooling, add 0.3 mL of phenolphthalein TS: the solution is colorless, and not more than 0.4 mL of 0.1 mol/L sodium hydroxide VS is required to produce a pale red color or red color.

*3*  Heavy metals <1.07>—Dissolve 4.0 g of Lactose Hydrate in 20 mL of warm water, add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make 50 mL. Proceed with this solution according to Method 1, and perform the test. Prepare the control solution with 1 mL of 0.1 mol/L hydrochloric acid TS and 2.0 mL of Standard Lead Solution (not more than 5 ppm).

(4)  Proteins and light absorbing substances—Dissolve 1.0 g of Lactose Hydrate in water to make 100 mL, and use this solution as the sample solution. Determine the absorbances as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control solution: not more than 0.25 at between 210 nm and 220 nm, and not more than 0.07 at between 270 nm and 300 nm.

*Loss on drying* <2.41>  Not more than 0.5%. For the granulated powder, not more than 1.0% (1 g, 80°C, 2 hours).

**Water** <2.48>  4.5–5.5%.  For the granulated powder, 4.0–5.5%  (1 g, volumetric titration, direct titration. Use a mixture of methanol for Karl Fischer method and formamide for Karl Fischer method (2:1) instead of methanol for Karl Fischer method).

**Residue on ignition** <2.44>  Not more than 0.1% (1 g).

*Microbial limit* <4.05>  The acceptance criteria of TACM and TYMC are 10^5 CFU/g and 5 × 10^4 CFU/g, respectively. *Salmonella* and *Escherichia coli* are not observed.

*Containers and storage*  Containers—Well-closed containers.
Lactulose

ラクトース

C_{12}H_{22}O_{11}: 342.30
β-D-Galactopyranosyl-(1→4)-d-fructose
[4618-18-2]

Lactulose is a solution of lactulose prepared by isomerizing lactose under the existing of alkaline and purified by ion-exchange resin.

It contains not less than 50.0% and not more than 56.0% of C_{12}H_{22}O_{11}.

Description  Lactulose occurs as a clear, colorless or light yellow, viscous liquid. It is odorless, and has a sweet taste.

It is miscible with water and with formamide.

Identification (1) To 0.7 g of Lactulose add 10 mL of water, 10 mL of a solution of hexaammomium heptamolybdate tetrahydrate (1 in 25) and 0.2 mL of acetic acid (100), and heat in a water bath for 5 to 10 minutes: a blue color develops.

(2) Mix 0.3 g of Lactulose and 30 mL of water, add 16 mL of 0.5 mol/L iodine TS, then immediately add 2.5 mL of 8 mol/L sodium hydroxide TS, allow to stand for 7 minutes, and add 2.5 mL of diluted sulfuric acid (3 in 20). To this solution add a saturated solution of sodium sulfite heptahydrate until the solution turns light yellow, then add 3 drops of methyl orange TS, neutralize with a solution of sodium hydroxide until the solution turns light yellow, then add 3 drops of methyl orange TS, neutralize with a solution of sodium hydroxide (4 in 25), and add water to make 100 mL. To 10 mL of this solution add a saturated solution of sodium sulfite heptahydrate until the solution turns light yellow, then add 3 drops of methyl orange TS, neutralize with a solution of sodium hydroxide (4 in 25), and add water to make 100 mL. To 10 mL of this solution add 5 mL of Fehling’s TS, and boil for 5 minutes: a red precipitate is produced.

pH <2.54> To 2.0 g of Lactulose add water to make 15 mL: the pH of the solution is between 3.5 and 5.5.

Specific gravity <2.56> $d_20^0$: 1.320 – 1.360

Purity (1) Heavy metals <1.07>—Proceed with 5.0 g of Lactulose according to Method 4, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(2) Arsenic <1.17>—Prepare the test solution with 1.0 g of Lactulose according to Method 1, and perform the test (not more than 2 ppm).

(3) Galactose and lactose—Determine the heights of the peaks corresponding to galactose and lactose respectively, on the chromatogram obtained in Assay from the sample solution and the standard solution, and calculate the ratios of the peak heights of galactose and lactose to that of the internal standard from the sample solution, $Q_{Ta}$ and $Q_{Tb}$, and then from the standard solution, $Q_{Sa}$ and $Q_{Sb}$: it contains galactose of not more than 11%, and lactose of not more than 6%.

$M_S$: Amount (mg) of lactose

Amount (mg) of lactose

\[ M_S = M_{Sb} \times Q_{Tb}/Q_{Sa} \]

$M_S$: Amount (mg) of lactose hydrate

Amount (mg) of lactose hydrate

\[ M_S = M_{Sb} \times Q_{Tb}/Q_{Sa} \]

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 1 g of Lactulose, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.5 g of Lactulose RS, accurately about 80 mg of β-galactose and accurately about 40 mg of lactose monohydrate, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak height of lactulose to that of the internal standard, respectively.

$M_S$: Amount (mg) of C_{12}H_{22}O_{11}$

\[ M_S = M_{Sb} \times Q_{Tb}/Q_{Sa} \]

Containers and storage  Containers—Tight containers.
Lanatoside C

ラナトシド C

C₉H₇₆O₂₀: 985.12
3β-[β-D-Glucopyranosyl-(1→4)-3-O-acetyl-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-ribo-hexopyranosylxylo]-12β,14-dihydroxy-5β,14β-card-20(22)-enolide

[17575-22-3]

Lanatoside C, when dried, contains not less than 90.0% and not more than 102.0% of C₉H₇₆O₂₀.

Description Lanatoside C occurs as colorless or white crystals or a white, crystalline powder. It is odorless.

It is soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It is hygroscopic.

Identification Place 1 mg of Lanatoside C to a small test tube having an internal diameter of about 10 mm, dissolve in 1 mL of a solution of iron (III) chloride hexahydrate in acetic acid (100) (1 in 10,000), and underlay gently with 1 mL of sulfuric acid: at the zone of contact of the two liquids, a brown ring is produced, and the color of the upper layer near the contact zone gradually changes to blue through purple. Finally the color of the entire acetic acid layer changes to blue-green through deep blue.

Purity Related substances—Dissolve 10 mg of Lanatoside C in exactly 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1.0 mg of Lanatoside C RS in exactly 5 mL of methanol, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography (2.03) with these solutions. Spot 20 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (84:15:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 110°C for 10 minutes: any spots other than the principal spot from the sample solution are neither larger nor darker than the spot from the standard solution.

Optical rotation <2.49> [α]D: +32° +35° (after drying, 0.5 g, methanol, 25 mL, 100 mm).

Loss on drying <2.41> Not more than 7.5% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Residue on ignition <2.44> Not more than 0.5% (0.1 g).

Assay Weigh accurately about 50 mg each of Lanatoside C and Lanatoside C RS, previously dried, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL each of these solutions, add methanol to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Pipet 5 mL each of the sample solution and standard solution into 25-mL light-resistant, volumetric flasks, and add 5 mL of 2,4,6-trinitrophenol TS and 0.5 mL of a solution of sodium hydroxide (1 in 10), shake well, and add methanol to make 25 mL. Allow these solutions to stand between 18°C and 22°C for 25 minutes, and determine the absorbances, A₁ and A₅, of the solutions at 485 nm as directed under Ultraviolet-visible Spectrophotometry (2.24), using a solution prepared with 5 mL of methanol in the same manner as the blank solution.

Amount (mg) of C₉H₇₆O₂₀ = Mₛ × A₁/A₅

Mₚ: Amount (mg) of Lanatoside C RS

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Lanatoside C Tablets

ラナトシド C錠

Lanatoside C Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of lanatoside C (C₉H₇₆O₂₀: 985.12).

Method of preparation Prepare as directed under Tablets, with Lanatoside C.

Identification (1) Shake a quantity of powdered Lanatoside C Tablets, equivalent to 1 mg of Lanatoside C according to the labeled amount, with 3 mL of diethyl ether, and filter. Wash the residue with two 3-mL portions of diethyl ether, and air-dry. To the remaining residue add 10 mL of a mixture of chloroform and methanol (9:1), shake, and filter. Wash the residue with two 5-mL portions of a mixture of chloroform and methanol (9:1), combine the filtrate and washings, and evaporate on a water bath to a smaller volume. Transfer the solution to a small test tube having an internal diameter of about 10 mm, further evaporate on a water bath to dryness, and proceed as directed in the Identification under Lanatoside C.

(2) Perform the test with the sample solution and the standard solution obtained in the Assay as directed under Thin-layer Chromatography (2.03). Spot 25 µL each of these solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (84:15:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 110°C for 10 minutes: the spots obtained from the sample solution and standard solution show a black color, and have the same Rf values.
Uniformity of dosage unit <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Warm 1 tablet of Lanatoside C Tablets with 5 mL of water until the tablet is disintegrated, add 30 mL of ethanol (95), disperse finely the particles with the aid of ultrasonic waves, add ethanol (95) to make exactly 50 mL of a solution containing about 25 mg of Lanatoside C (C₄₉H₇₆O₂₀) in each mL, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Lanatoside C RS, previously dried in vacuum over phosphorus (V) oxide at 60°C for 4 hours, and dissolve in ethanol (95) to make exactly 100 mL. Pipet 2 mL of this solution, add 10 mL of water, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Pipet 2 mL of each of the sample solution, the standard solution and diluted ethanol (95) (17 in 20) into three brown glass-stoppered test tubes T, S and B, previously containing exactly 10 mL of 0.012 w/v L-ascorbic acid-hydrochloric acid TS, add exactly 1 mL each of dilute hydrogen peroxide TS immediately, shake vigorously, and allow to stand at a constant temperature between 25°C and 30°C for 40 minutes. Determine the fluorescence intensities, \( F_T \), \( F_S \) and \( F_B \), of the subsequent solutions from the sample solution and the standard solution and the diluted ethanol (95) (17 in 20) at 355 nm of the excitation wavelength and at 490 nm of the fluorescence wavelength as directed under Fluorometry <2.22>, respectively.

\[
\text{Amount (mg) of lanatoside C (C₄₉H₇₆O₂₀)} = M_S \times \frac{(F_T - F_B)}{(F_S - F_B)} \times \frac{V}{5000}
\]

\( M_S \): Amount (mg) of Lanatoside C RS

Dissolution <6.10> When the test is performed at 100 revolutions per minute according to the Paddle method, using 500 mL of distilled hydrochloric acid (3 in 500) as the dissolution medium, the dissolution rate in 60 minutes of Lanatoside C Tablets is not less than 65%. No retest requirement is applied to Lanatoside C Tablets.

Start the test with 1 tablet of Lanatoside C Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 \( \mu \)m. Discard the first 10 mL of the filtrate, pipet \( V \) mL of the subsequent filtrate, add the dissolution medium to make exactly \( V \) mL so that each mL contains about 0.5 \( \mu \)g of lanatoside C (C₄₉H₇₆O₂₀) according to the labeled amount, and use this solution as the sample solution. Separately, dry Lanatoside C RS in vacuum over phosphorus (V) oxide at 60°C for 4 hours, weigh accurately a portion of it, equivalent to 100 times an amount of the labeled amount of lanatoside C (C₄₉H₇₆O₂₀), dissolve in ethanol (95) to make exactly 100 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 500 mL, warm at 37 ± 0.5°C for 60 minutes, and use this solution as the standard solution. Pipet 3 mL each of the sample solution, the standard solution and the dissolution medium, and transfer to glass-stoppered brown test tubes T, S and B, respectively. To these solutions add exactly 10 mL each of 0.012 w/v L-ascorbic acid-hydrochloric acid TS, and shake. Immediately add exactly 0.2 mL each of dilute hydrogen peroxide TS (1 in 100), shake well, and allow to stand at a constant temperature between 30°C and 37°C for 45 minutes. Determine immediately the fluorescence intensities, \( F_T \), \( F_S \) and \( F_B \), of the sample solution and the standard solution at 355 nm of the excitation wavelength and at 490 nm of the fluorescence wavelength as directed under Fluorometry <2.22>.

Dissolution rate (%) with respect to the labeled amount of lanatoside C (C₄₉H₇₆O₂₀)

\[
M = M_S \times \frac{(F_T - F_B)}{(F_S - F_B)} \times \frac{V}{V_5/1/C}
\]

\( M \): Amount (mg) of Lanatoside C RS

C: Labeled amount (mg) of lanatoside C (C₄₉H₇₆O₂₀) in 1 tablet

Assay Weigh accurately and powder not less than 20 Lanatoside C Tablets. Weigh accurately a portion of the powder, equivalent to about 5 mg of lanatoside C (C₄₉H₇₆O₂₀), into a 100-mL light-resistant volumetric flask, add 50 mL of ethanol (95), and shake for 15 minutes. Then dilute with ethanol (95) to make exactly 100 mL. Filter this solution, discard the first 20 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 5 mg of Lanatoside C RS, previously dried in vacuum over phosphorus (V) oxide at 60°C for 4 hours, dissolve in ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution into light-resistant, glass-stoppered test tubes, add exactly 3 mL each of alkaline 2,4,6-trinitrophenol TS, shake well and allow these solutions to stand between 22°C and 28°C for 25 minutes. Determine the absorbances, \( A_T \) and \( A_S \), of the subsequent sample solution and the subsequent standard solution at 490 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared by the same manner with 5 mL of ethanol (95), as the blank.

\[
\text{Amount (mg) of lanatoside C (C₄₉H₇₆O₂₀)} = M_S \times \frac{A_T}{A_S}
\]

\( M_S \): Amount (mg) of Lanatoside C RS

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Hydrous Lanolin 加水ラノリン

Hydrous Lanolin is Purified Lanolin to which water is added. It contains not less than 70% and not more than 75% of Purified Lanolin (as determined by the test for Residue on evaporation).

Description Hydrous Lanolin is a yellowish white, ointment-like substance, and has a slight, characteristic odor, which is not rancid.

It is soluble in diethyl ether and in cyclohexane, with the separation of water.

When melted by heating on a water bath, it separates into a clear oily layer and a clear water layer.

Melting point: about 39°C.

Identification Dissolve 1 g of Hydrous Lanolin in 50 mL of cyclohexane, and remove the separated water. Superimpose carefully 1 mL of the cyclohexane solution on 2 mL of sulfuric acid: a red-brown color develops at the zone of contact,
and sulfuric acid layer shows a green fluorescence.

Acid value <1.13> Not more than 1.0.

Iodine value 18 – 36 Heat a suitable amount of Hydrous Lanolin on a water bath to remove its almost moisture, then weigh accurately about 0.8 g of the treated Hydrous Lanolin in a glass-stoppered 500-mL flask, and add 10 mL of cyclohexane to dissolve, and add exactly 25 mL of Hanus's TS, and mix well. If a clear solution is not obtained, add more cyclohexane to make clear, and allow the mixture to stand for 1 hour between 20°C and 30°C in a light-resistant, well-closed container while occasional shaking. Add 20 mL of a solution of potassium iodide (1 in 10) and 100 mL of water, shake, and titrate with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner.

\[ \text{Iodine value} = (a - b) \times 1.269/M \]

M: amount (g) of sample
a: Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed in the blank determination
b: Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed in the titration

Purity (1) Acidity or alkalinity—To 5 g of Hydrous Lanolin add 25 mL of water, boil for 10 minutes, and cool. Add water to restore the previous mass, and separate the aqueous layer: the aqueous layer is neutral.

(2) Chloride <1.07>—To 2.0 g of Hydrous Lanolin add 40 mL of water, boil for 10 minutes, and cool. Add water to restore the previous mass, and filter. To 20 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Use this solution as the test solution, and perform the test. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(3) Ammonia—To 10 mL of the aqueous layer obtained in (1) add 1 mL of sodium hydroxide TS, and boil: the gas evolved does not turn moistened red litmus paper to blue.

(4) Water-soluble organic substances—To 5 mL of the aqueous layer obtained in (1) add 0.25 mL of 0.002 mol/L potassium permanganate VS, and allow to stand for 5 minutes: the red color of the solution does not disappear.

(5) Petrolatum—Dissolve 1.0 g of the dried residue obtained in the Residue on evaporation in 50 mL of a mixture of tetrahydrofuran and isooctane (1:1), and use this solution as the sample solution. Add dissolve 20 mg of vaseline in 10 mL of a mixture of tetrahydrofuran and isooctane (1:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 25 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with isooctane to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 2) on the plate, heat the plate at 80°C for 5 minutes, cool, and examine under ultraviolet light (main wavelength: 365 nm): no fluorescent spot is observed in the same level with the spot of standard solution. For this test use a thin-layer plate previously developed with isooctane to the upper end, dried in air, and heated at 110°C for 60 minutes.

Residue on evaporation Weigh accurately about 12.5 g of Hydrous Lanolin, dissolve in 50 mL of diethyl ether, place it in a separator, transfer the separated aqueous layer to another separator, add 10 mL of diethyl ether, shake, and combine the diethyl ether layer and diethyl ether in the first separator. Shake the diethyl ether layer with 3 g of anhydrous sodium sulfate, and filter through dry filter paper. Wash the separator and the filter paper with two 20-mL portions of diethyl ether, combine the washings with the filtrate, evaporate on a water bath until the odor of diethyl ether is no longer perceptible, and dry in a desiccator (in vacuum, silica gel) for 24 hours: the content is not less than 70% and not more than 75%.

Containers and storage Containers—Well-closed containers.

Storage—Not exceeding 30°C.

**Purified Lanolin**

*Adeps Lanae Purificatus*

精製ラノリン

Purified Lanolin is the purified product of the fat-like substance obtained from the wool of *Ovis aries* Linne (Bovidae).

**Description** Purified Lanolin is a light yellow to yellowish brown, viscous, ointment-like substance, and has a faint, characteristic but not rancid odor.

It is very soluble in diethyl ether and in cyclohexane, freely soluble in tetrahydrofuran and in toluene, and very slightly soluble in ethanol (95). It is practically insoluble in water, but miscible without separation with about twice its mass of water, retaining ointment-like viscosity.

Melting point: 37 – 43°C

**Identification** Superimpose carefully 1 mL of a solution of Purified Lanolin in cyclohexane (1 in 50) on 2 mL of sulfuric acid: a red-brown color develops at the zone of contact, and the sulfuric acid layer shows a green fluorescence.

Acid value <1.13> Not more than 1.0.

Iodine value 18 – 36 Weigh accurately about 0.8 g of Purified Lanolin in a glass-stoppered 500-mL flask, add 20 mL of cyclohexane to dissolve, and add exactly 25 mL of Hanus's TS, and mix well. If a clear solution is not obtained, add more cyclohexane to make clear, and allow the mixture to stand for 1 hour between 20°C and 30°C in light-resistant, well-closed containers, with occasional shaking. Add 20 mL of a solution of potassium iodide (1 in 10) and 100 mL of water, shake, and titrate with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

\[ \text{Iodine value} = (a - b) \times 1.269/M \]

M: amount (g) of sample
a: Volume (mL) of 0.1 mol/L sodium thiosulfate VS used in the blank determination
b: Volume (mL) of 0.1 mol/L sodium thiosulfate VS used in the titration of the sample.

Purity (1) Acid or alkali—To 5 g of Purified Lanolin add 25 mL of water, boil for 10 minutes, and cool. Add water to restore the previous mass, and separate the aqueous layer:
Lard / Official Monographs

Purity (1) Moisture and coloration—Melt 5 g of Lard by heating on a water bath: it forms a clear liquid, from which no water separates. Observe the liquid in a layer 10 mm thick: the liquid is colorless to slightly yellow.

(2) Alkalinity—To 2.0 g of Lard add 10 mL of water, melt by warming on a water bath, and shake vigorously. After cooling, add 1 drop of phenolphthalein TS to the separated water layer: the layer is colorless.

(3) Chloride <0.05—To 1.5 g of Lard add 30 mL of ethanol (95), boil for 10 minutes under a reflux condenser, and filter after cooling. To 20 mL of the filtrate add 5 drops of a solution of silver nitrate in ethanol (95) (1 in 50): the opalescence of the mixture does not exceed that of the following control solution.

Control solution: To 1.0 mL of 0.01 mol/L hydrochloric acid VS add ethanol (95) to make 20 mL, and add 5 drops of a solution of silver nitrate in ethanol (95) (1 in 50).

(4) Beef tallow—Dissolve 5 g of Lard in 20 mL of diethyl ether, stopper lightly with absorbent cotton, and allow to stand at 20°C for 18 hours. Collect the separated crystals, moisten them with ethanol (95), and examine under a microscope of 200 magnifications: the crystals are in the form of rhomboidal plates grouped irregularly, and do not contain prisms or needles grouped in fan-shaped clusters.

Containers and storage Containers—Well-closed containers.

Storage—Not exceeding 30°C.

Latamoxef Sodium

ラタモキセフナトリウム

Latamoxef Sodium contains not less than 830 μg (potency) and not more than 940 μg (potency) per mg, calculated on the anhydrous basis. The potency of Latamoxef Sodium is expressed as mass (potency) of latamoxef (C_{20}H_{30}N_{4}Na_{2}O_{9}S: 520.47)

Description Latamoxef Sodium occurs as white to light yellowish white, powder or masses.

It is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Latamoxef Sodium (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.
(2) Determine the infrared absorption spectrum of Latamoxef Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2,25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavenumber.

(3) Determine the H spectrum of a solution of Latamoxef Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2,21>, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A and B, at around δ 3.5 ppm and at around δ 4.0 ppm. The ratio of the integrated intensity of these signals, A:B, is about 1:1.

(4) Latamoxef Sodium responds to the Qualitative Tests <1,09> (1) for sodium salt.

Optical rotation <2,49> [α]20D = -32° – -40° (0.5 g calculated on the anhydrous basis, phosphate buffer solution, pH 7.0, 50 mL, 100 mm).

pH <2,52> The pH of a solution obtained by dissolving 1.0 g of Latamoxef Sodium in 10 mL of water is between 5.0 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Latamoxef Sodium in 10 mL of water: the solution is clear and has no more color than the following control solution.

Control solution: To a mixture of 3.0 mL of Cobalt (II) Chloride CS and 36 mL of Iron (III) Chloride CS add 11 mL of diluted dilute hydrochloric acid (1 in 10). To 2.5 mL of this solution add 7.5 mL of diluted dilute hydrochloric acid (1:10).

(2) Heavy metals <1,07>—Carbonize 1.0 g of Latamoxef Sodium by heating gently, previously powdered if it is masses. After cooling, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (1 in 10), and burn the ethanol. After cooling, add 1 mL of sulfuric acid. Proceed according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1,14>—Prepare the test solution by dissolving 1.0 g of Latamoxef Sodium in 20 mL of water, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve an amount of Latamoxef Sodium, equivalent to about 25 mg (potency), in water to make exactly 50 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2,01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of 1-methyl-1H-tetrazole-5-thiol, having the relative retention time of about 0.5 with respect to the first eluted peak of the two peaks of latamoxef, obtained from the sample solution is not less than the peak area of latamoxef from the standard solution, and the peak area of decarboxyllatamoxef, having the relative retention time of about 1.7 with respect to the first peak of the two peaks of latamoxef, is not larger than 2 times that of latamoxef from the standard solution. For this calculation, use the peak area for 1-methyl-1H-tetrazole-5-thiol after multiplying by its relative response factor, 0.52.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 5 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of latamoxef is not more than 2.0%.

Water <2,48> Not more than 5.0% (0.5 g, volumetric titration, back titration).

Isomer ratio Dissolve 25 mg of Latamoxef Sodium in water to make 50 mL, and use this solution as the sample solution. Perform the test with 5 µL of the sample solution as directed under Liquid Chromatography <2,01> according to the following conditions, and determine the areas, A1 and A2, of the two peaks in order of elution, which appear close to each other at the retention time of about 10 minutes: A1/A2 is between 0.8 and 1.4.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 7.7 g of ammonium acetate in water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of the first eluted peak of latamoxef is about 8 minutes.

System suitability—

System performance: When the procedure is run with 5 µL of the sample solution under the above operating conditions, the resolution between the two peaks of latamoxef is not less than 3.

System repeatability: When the test is repeated 3 times with 5 µL of the sample solution under the above operating conditions, the relative standard deviation of the area of the first eluted peak of latamoxef is not more than 2.0%.

Assay Weigh accurately an amount of Latamoxef Sodium and Latamoxef Ammonium RS, equivalent to about 25 mg (potency) each, dissolve in exactly 5 mL of the internal standard solution, add water to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2,01> according to the following conditions, and calculate the ratios, Q1 and Q2, of the peak area of latamoxef to that of the internal standard.

Amount [µg (potency)] of latamoxef (C20H20N6O9S) = \( M_5 \times \frac{Q_1}{Q_2} \times 1000 \)  

\( M_5 \): Amount [mg (potency)] of Latamoxef Ammonium RS

Internal standard solution—A solution of m-cresol (3 in 200).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 6.94 g of potassium dihydrogen phosphate, 3.22 g of disodium hydrogen phosphate dodecahydrate and 1.60 g of tetra n-butylammonium bromide in water to make exactly 1000 mL. To 750 mL of this solution add 250 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of latamoxef is about 7 minutes.
System suitability—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, latamoxef and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of latamoxef to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Not exceeding 5°C.

Laumacroglol
Polyoxyethylene Lauryl Alcohol Ether
ラウロマクロゴール

Laumacroglol is a polyoxyethylene ether prepared by the polymerization of ethylene oxide with laury alcohol.

Description Laumacroglol is a colorless or light yellow, clear liquid or a white, petrolatum-like or waxy solid. It has a characteristic odor, and a somewhat bitter and slightly irritative taste.

It is very soluble in water, in methanol and in ethanol (95), and freely soluble in N,N-dimethylformamide.

Identification (1) Shake well 0.5 g of Lauromacrogol with 10 mL of water and 5 mL of ammonium thiocyanate-cobalt nitrate TS, then shake with 5 mL of chloroform, and allow to stand: the chloroform layer becomes blue in color.

(2) Dissolve 0.35 g of Lauromacrogol in 10 mL of carbon tetrachloride, and perform the test as directed in the Solution method under Infrared Spectrophotometry <2.25> using a 0.1-mm fixed cell: it exhibits absorption at the wave numbers of about 1347 cm⁻¹, 1246 cm⁻¹ and 1110 cm⁻¹.

Purity (1) Acidity—Transfer 10.0 g of Lauromacrogol into a flask, and add 50 mL of neutralized ethanol. Heat on a water bath nearly to boil, shaking once or twice while heating. Cool, and add 5.3 mL of 0.1 mol/L sodium hydroxide VS and 5 drops of phenolphthalein TS: a red color develops.

Lenampicillin Hydrochloride


C₅₂H₇₂N₄O₅S.HCl: 497.95
5-Methyl-2-oxo-[1,3]dioxol-4-ylmethyl (2S,5R,6R)-6-[(2R)-2-amino-2-phenylacetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrochloride [80734-02-7]

Lenampicillin Hydrochloride is the hydrochloride of ampicillin methylthiodioxolylethyl ester.
It contains not less than 653 μg (potency) and not more than 709 μg (potency) per mg, calculated on the anhydrous basis and corrected by the amount of the residual solvents. The potency of Lenampicillin Hydrochloride is expressed as mass (potency) of ampicillin (C₁₆H₁₉N₃O₄S: 349.40).

Description Lenampicillin Hydrochloride occurs as a white to light yellowish white powder.
It is very soluble in water, in methanol and in ethanol (95), and freely soluble in N,N-dimethylformamide.

Identification (1) Determine the infrared absorption spectrum of Lenampicillin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Lenampicillin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) To 1 mL of a solution of Lenampicillin Hydrochloride (1 in 100) add 0.5 mL of dilute nitric acid and 1 drop of silver nitrate TS: a white precipitate is formed.

Optical rotation <2.49> [α]D₂⁰: +174° to +194° (0.2 g calculated on the anhydrous basis and corrected on the amount of residual solvent, ethanol (95), 20 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Lenampicillin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.17>—Prepare the test solution with 1.0 g of Lenampicillin Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(3) Free ampicillin—Weigh accurately about 0.1 g of Lenampicillin Hydrochloridein, dissolve in exactly 10 mL of the internal standard solution, and use this solution as the
sample solution. Separately, weigh accurately an amount of Ampicillin RS, equivalent to about 25 mg (potency), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. The sample solution should be used to the following test immediately after the solution is prepared. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_s \) and \( Q_a \), of the peak height of ampicillin to that of the internal standard:

the amount of ampicillin is not more than 1.0%.

\[
\text{Amount} (\%) \text{ of ampicillin} \ (C_{16}H_{21}N_3O_5S) = \frac{M_S}{M_T} \times \frac{Q_a}{Q_s} \times 2
\]

\( M_S \): Amount (mg [potency]) of Ampicillin RS
\( M_T \): Amount (mg) of the sample

Internal standard solution—A solution of anhydrous caffeine in the mobile phase (1 in 50,000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 230 nm).
Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadeclsilylanized silica gel for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 1.22 g of potassium dihydrogen phosphate in water to make 900 mL, and add 100 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of ampicillin is about 7 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ampicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of ampicillin to that of the internal standard is not more than 5%.

(4) Penicilloic acid—Weigh accurately about 0.1 g of Lenampicillin Hydrochloride, dissolve in water to make exactly 100 mL, and use this solution as the sample solution.

Pipet 10 mL of the sample solution, add 10 mL of potassium hydrogen phthalate buffer solution, pH 4.6 and exactly 10 mL of 0.005 mol/L iodine VS, allow to stand for exactly 15 minutes while protecting from exposure to light, and titrate <2.50> with 0.01 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination, and make any necessary correction: the amount of penicilloic acid (\( C_{16}H_{21}N_3O_5S: 367.42 \)) is not more than 3.0%.

Each mL of 0.01 mol/L sodium thiosulfate VS = 0.45 mg of \( C_{16}H_{21}N_3O_5S \)

(5) Residual solvent <2.46>—Weigh accurately about 0.25 g of Lenampicillin Hydrochloride, dissolve in exactly 1 mL of the internal standard solution, add \( N,N \)-dimethylformamide to make 5 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of 2-propanol and about 0.12 g of ethyl acetate, and add \( N,N \)-dimethylformamide to make exactly 100 mL. Pipet 1 mL and 3 mL of this solution, add exactly 1 mL each of the internal standard solution, add \( N,N \)-dimethylformamide to make 5 mL, and use these solutions as the standard solution (1) and the standard solution (2), respectively. Perform the test with 4 μL each of the sample solution, standard solution (1) and (2) as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, \( Q_s \) and \( Q_a \), of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the sample solution, the ratios, \( Q_{s01} \) and \( Q_{a01} \), of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the standard solution (1) and the ratios, \( Q_{s02} \) and \( Q_{a02} \), of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the standard solution (2). Calculate the amounts of 2-propanol and ethyl acetate by the following equations: not more than 0.7% and not more than 1.7%, respectively.

\[
\text{Amount} (\%) \text{ of 2-propanol} = \frac{M_{s0}/M_T}{(2Q_{a01} - 3Q_{s01} + Q_{s02})/(Q_{s02} - Q_{s01})}
\]

\[
\text{Amount} (\%) \text{ of ethyl acetate} = \frac{M_{s0}/M_T}{(2Q_{a02} - 3Q_{s02} + Q_{s01})/(Q_{s02} - Q_{s01})}
\]

\( M_{s0} \): Amount (g) of 2-propanol
\( M_{a0} \): Amount (g) of ethyl acetate
\( M_T \): Amount (g) of the sample

Internal standard solution—A solution of cyclohexane in \( N,N \)-dimethylformamide (1 in 1000).

Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A glass column 3 mm in inside diameter and 3 m in length, packed with siliceous earth for gas chromatography (180 – 250 μm in particle diameter) coated with tetrahydroxypropylethylenediamine for gas chromatography at the ratio of 10 to 15%.
Column temperature: A constant temperature of about 80°C.
Injection port temperature: A constant temperature of about 160°C.
Carrier gas: Nitrogen.
Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 1 minute.

System suitability—
System performance: When the procedure is run with 4 μL of the standard solution (2) under the above operating conditions, the internal standard, ethyl acetate and 2-propanol are eluted in this order, and the resolution between the peaks of the internal standard and ethyl acetate is not less than 2.0.
System repeatability: When the test is repeated 3 times with 4 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the ratios of the peak height of ethyl acetate to that of the internal standard is not more than 5.0%.

Water <2.48> Not more than 1.5% (1 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately an amount of Lenampicillin Hydrochloride and Lenampicillin Hydrochloride RS, equivalent to about 0.1 g (potency), dissolve each in the internal stand-
ard solution to make exactly 10 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography $\leq 2.01\leq$ according to the following conditions, and calculate the ratios, $Q_1$ and $Q_3$, of the peak area of lenampicillin to that of the internal standard.

$$\text{Amount [μg (potency)] of ampicillin (C₁₆H₁₉N₃O₄S)} = M_S \times Q_1 / Q_3 \times 1000$$

$$M_S: \text{Amount [mg (potency)] of Lenampicillin Hydrochloride RS}$$

**Internal standard solution**—A solution of ethyl aminobenzolate in the mobile phase (1 in 4000).

**Operating conditions**—

**Detector:** An ultraviolet absorption photometer (wavelength: 254 nm).

**Column:** A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecysilanized silica gel for liquid chromatography (5 μm in particle diameter).

**Column temperature:** A constant temperature of about 25°C.

**Mobile phase:** Dissolve 9.53 g of potassium dihydrogen phosphate in water to make exactly 700 mL, and add acetonitrile to make exactly 1000 mL.

**Flow rate:** Adjust the flow rate so that the retention time of lenampicillin is about 6 minutes.

**System suitability**—

**System performance:** When the procedure is run with 5 μL of the standard solution under the above operating conditions, lenampicillin and the internal standard are eluted in the same wave numbers.

**System repeatability:** When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of lenampicillin to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.

### L-Leucine

L-ロイシン

\[ \text{C}_6\text{H}_{13}\text{NO}_2: 131.17 \]

(2S)-2-Amino-4-methylpentanoic acid

[61-90-5]

L-Leucine, when dried, contains not less than 98.5% of C₆H₁₃NO₂.

**Description**—L-Leucine occurs as white crystals or crystalline powder. It is odorless or has a faint characteristic odor, and has a slightly bitter taste.

It is freely soluble in formic acid, sparingly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

**Identification**—Determine the infrared absorption spectrum of L-Leucine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $\leq 2.25\leq$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation**—$\leq 2.49\leq$ [α]D$^0\leq +14.5$ – $+16.0^\circ$ (after drying, 1 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

**pH**—$\leq 2.54\leq$ Dissolve 1.0 g of L-Leucine in 100 mL of water: the pH of this solution is between 5.5 and 6.5.

**Purity (1)**—Clarity and color of solution—Dissolve 0.5 g of L-Leucine in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride $\leq 1.03\leq$—Dissolve 0.5 g of L-Leucine in 40 mL of water and 6 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate $\leq 0.02\leq$—Prepare the test solution with 0.10 g of L-Leucine in 40 mL of water and 1 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium $\leq 0.02\leq$—Prepare the test with 0.25 g of L-Leucine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals $\leq 2.0\leq$—Proceed with 1.0 g of L-Leucine according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic $\leq 1.1\leq$—Prepare the test solution with 1.0 g of L-Leucine according to Method 2, and perform the test (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of L-Leucine in water by warming, after cooling, add water to make 25 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\leq 2.0\leq$. Spot 5 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of l-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying**—$\leq 2.4\leq$ Not more than 0.30% (1 g, 105°C, 3 hours).

**Residue on ignition**—$\leq 2.4\leq$ Not more than 0.1% (1 g).

**Assay**—Weigh accurately about 0.13 g of L-Leucine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate $\leq 2.50\leq$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

$$= 13.12 \text{ mg of C}_6\text{H}_{13}\text{NO}_2$$

**Containers and storage**—Containers—Well-closed contain-
Levallorphan Tartrate

**Levallorphan Tartrate**

![Chemical Structure](image)

C_{19}H_{25}NO.C_4H_6O_6: 433.49

17-Allylmorphinan-3-ol monotartrate [71-82-9]

Levallorphan Tartrate, when dried, contains not less than 98.5% of C_{19}H_{25}NO.C_4H_6O_6.

**Description** Levallorphan Tartrate occurs as a white to pale yellow, crystalline powder. It is odorless.

It is soluble in water and in acetic acid (100), sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

**Identification**

1. **Determination** of the absorption spectrum of a solution of Levallorphan Tartrate in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

2. **Determine the infrared absorption spectrum of** Levallorphan Tartrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

3. A solution of Levallorphan Tartrate (1 in 30) responds to the Qualitative Tests <1.09> (1) and (2) for tartrate.

**Optical rotation** <2.49> [α]_D^20: −37.0° to −39.2° (after drying, 0.2 g, water, 10 mL, 100 mm).

**pH** <2.54> Dissolve 0.2 g of Levallorphan Tartrate in 20 mL of water: the pH of this solution is between 3.3 and 3.8.

**Melting point** <2.60> 174 – 178°C

**Purity**

1. **Clarity and color of solution**—Dissolve 0.2 g of Levallorphan Tartrate in 10 mL of water: the solution is clear and colorless.

2. **Heavy metals** <1.07>—Proceed with 1.0 g of Levallorphan Tartrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

3. **Related substances**—Dissolve 0.2 g of Levallorphan Tartrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia TS (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff’s TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus(V) oxide, 80°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.10% (1 g).

**Assay** Weigh accurately about 0.5 g of Levallorphan Tartrate, previously dried, dissolve in 30 mL of acetic acid (100), and titrate <2.25> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 43.35 mg of C_{19}H_{25}NO.C_4H_6O_6

**Containers and storage** Containers—Well-closed containers.

**Levallorphan Tartrate Injection**

**Levallorphan** Tartrate Injection is an aqueous solution for injection.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of levallorphan tartrate (C_{19}H_{25}NO.C_4H_6O_6: 433.49).

**Method of preparation** Prepare as directed under Injection, with Levallorphan Tartrate.

**Description** Levallorphan Tartrate Injection is a clear, colorless liquid.

**pH** 3.0 – 4.5

**Identification** Take an exact volume of Levallorphan Tartrate Injection, equivalent to 3 mg of Levallorphan Tartrate according to the labeled amount, add 5 mL of water and 2 drops of dilute hydrochloric acid, and wash with five 15-mL portions of diethyl ether by a vigorous shaking. Take the water layer, evaporate the diethyl ether remaining by warming on a water bath, and after cooling, add 0.01 mol/L hydrochloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 277 nm and 281 nm.

**Bacterial endotoxins** <4.01> Less than 150 EU/mg.

**Extractable volume** <6.05> It meets the requirement.

**Foreign insoluble matter** <6.06> Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Take exactly a volume of Levallorphan Tartrate Injection, equivalent to about 2 mg of levallorphan tartrate (C_{19}H_{25}NO.C_4H_6O_6), add exactly 10 mL of the internal
Levodopa

**Levodopa**

\[ \text{C}_9\text{H}_11\text{NO}_4: \ 197.19 \]

3-Hydroxy-L-tyrosine

\[ [\alpha]_l^{20} = -11.5 \text{ to } -13.0^\circ \]

Levodopa, when dried, contains not less than 98.5% of \( \text{C}_9\text{H}_11\text{NO}_4 \).

**Description**

Levodopa occurs as white or slightly grayish white crystals or crystalline powder. It is odorless.

It is freely soluble in formic acid, slightly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

The pH of a saturated solution of Levodopa is between 5.0 and 6.5.

Melting point: about 275°C (with decomposition).

**Identification**

1. To 5 mL of a solution of Levodopa (1 in 1000) add 1 mL of ninhydrin TS, and heat for 3 minutes in a water bath: a purple color develops.

2. To 2 mL of a solution of Levodopa (1 in 5000) add 10 mL of 4-aminophthalazine TS, and shake: a red color develops.

3. Dissolve 3 mg of Levodopa in 0.001 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry \( \text{C}_2\text{H}_4\text{O} \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Optical rotation**

\[ [\alpha]_l^{20} = -11.5 \text{ to } -13.0^\circ \]

**Purity**

1. Clarity and color of solution—Dissolve 1.0 g of Levodopa in 20 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

2. Chloride—Dissolve 0.5 g of Levodopa in 6 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.3 mL of 0.001 mol/L hydrochloric acid VS (not more than 0.021%).

3. Sulfate—Dissolve 0.40 g of Levodopa in 1 mL of dilute hydrochloric acid and 30 mL of water, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.005 mol/L sulfuric acid VS (not more than 0.030%).

4. Heavy metals—Proceed with 1.0 g of Levodopa according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

5. Arsenic—Dissolve 1.0 g of Levodopa in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).

6. Related substances—Dissolve 0.10 g of Levodopa in 10 mL of sodium disulfite TS, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add sodium disulfite TS to make exactly 25 mL. Pipet 1 mL of this solution, add sodium disulfite TS to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( \text{C}_2\text{H}_4\text{O} \). Spot 5 \( \mu \)L each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, acetic acid (100) and methanol (10:5:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate and heat at 90°C for 10 minutes; the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.
Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Levodopa, previously dried, dissolve in 3 mL of formic acid, add 80 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 19.72 mg of C7H7NO4

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Levofloxacin Hydrate

レボフロキサシン水和物

C18H20FN3O4 \( \cdot \) H2O: 370.38

(3S)-9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7\(\text{H}\)-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid hemihydrate

[138199-71-0]

Levofloxacin Hydrate contains not less than 99.0% and not more than 101.0% of levofloxacin (C18H20FN3O4: 361.37), calculated on the anhydrous basis.

Description Levofloxacin Hydrate occurs as light yellowish white to yellowish white crystals or crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in water and in methanol, and slightly soluble in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

It gradually turns dark light yellowish white on exposure to light.

Melting point: about 226°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Levofloxacin Hydrate in 0.1 mol/L hydrochloric acid solution (1 in 150,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Levofloxacin Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> \( [\alpha]_D^20 \) = -92 – -99° (0.1 g calculated on the anhydrous basis, methanol, 10 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Levofloxacin Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 50 mg of Levofloxacin Hydrate in 10 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and methanol (1:1) to make exactly 10 mL. Pipet 1 mL of this solution, add a mixture of water and methanol (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak having the relative retention time of about 1.2 with respect to levofloxacin obtained from the sample solution is not larger than 2/5 times the peak area of levofloxacin from the standard solution, and the area of each peak other than the peak of levofloxacin and other than the peak having the relative retention time of about 1.2 with respect to levofloxacin from the sample solution is not larger than 1/5 times the peak area of levofloxacin from the standard solution. Furthermore, the total area of the peaks other than the peak of levofloxacin and other than the peak having the relative retention time of about 1.2 with respect to levofloxacin from the sample solution is not larger than 3/10 times the peak area of levofloxacin from the standard solution.

Operating conditions—


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: Dissolve 1.76 g of L-valine, 7.71 g of ammonium acetate and 1.25 g of Copper (II) sulfate pentahydrate in water to make 1000 mL. To this solution add 250 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of levofloxacin is about 22 minutes.

Time span of measurement: About 2 times as long as the retention time of levofloxacin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of water and methanol (1:1) to make exactly 20 mL. Confirm that the peak area of levofloxacin obtained from 10 µL of this solution is equivalent to 4 to 6% of that of levofloxacin from 10 µL of the standard solution.

System performance: Dissolve 10 mg of ofloxacin in 20 mL of a mixture of water and methanol (1:1). To 1 mL of this solution add a mixture of water and methanol (1:1) to make exactly 10 mL. When the procedure is run with 10 µL of this solution under the above operating conditions, the resolution between the peak of levofloxacin and the peak having the relative retention time of about 1.2 with respect to levofloxacin is not less than 3.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operat-
ing conditions, the relative standard deviation of the peak area of levofloxacin is not more than 3.0%.

(3) Residual solvent Being specified separately.

Water \(<2.48\) 2.1 – 2.7% (0.5 g, volumetric titration, direct titration).

Residue on ignition \(<2.44\) Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Levofloxacin Hyethanol (95) and in acetone, very slightly soluble in water, proform, sparingly soluble in methanol, slightly soluble in\(\text{tween 124}\) not less than 98.0% (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 36.14 mg of \(\text{C}_{19}\text{H}_{24}\text{N}_{2}\text{OS}\cdot\text{C}_{4}\text{H}_{4}\text{O}_{4}\)

Containers and storage Containers—Tight containers.

Levomepromazine Maleate

レボメプロマジンマレイン酸塩

![Chemical structure of Levomepromazine Maleate](image)

\(\text{C}_{19}\text{H}_{24}\text{N}_{2}\text{OS}\cdot\text{C}_{4}\text{H}_{4}\text{O}_{4}\) 444.54

(2\(R\))-(2-Methoxy-10\(H\)-phenothiazin-10-yl)-\(N, N, 2\)-trimethylpropylamine monomaleate [7104-38-3]

Levomepromazine Maleate, when dried, contains not less than 98.0% of \(\text{C}_{19}\text{H}_{24}\text{N}_{2}\text{OS}\cdot\text{C}_{4}\text{H}_{4}\text{O}_{4}\).

Description Levomepromazine Maleate occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in acetic acid (100), soluble in chloroform, sparingly soluble in methanol, slightly soluble in ethanol (95) and in acetone, very slightly soluble in water, and practically insoluble in diethyl ether.

Melting point: 184 – 190°C (with decomposition).

Identification (1) Dissolve 5 mg of Levomepromazine Maleate in 5 mL of sulfuric acid: a red-purple color develops, which slowly becomes deep red-purple. To this solution add 1 drop of potassium dichromate TS: a brownish yellow is produced.

(2) To 0.2 g of Levomepromazine Maleate add 5 mL of sodium hydroxide TS and 20 mL of diethyl ether, and shake well. Separate the diethyl ether layer, wash twice with 10-mL portions of water, add 0.5 g of anhydrous sodium sulfate, filter, evaporate the diethyl ether on a water bath, and dry the residue at 105°C for 2 hours: the residue melts \(<2.60\) between 124°C and 128°C.

(3) To 0.5 g of Levomepromazine Maleate add 5 mL of water and 2 mL of ammonia solution (28), extract with three 5-mL portions of chloroform, separate and evaporate the water layer to dryness. To the residue add 2 to 3 drops of dilute sulfuric acid and 5 mL of water, and extract with four 25-mL portions of diethyl ether. Combine all the diethyl ether extracts, evaporate the diethyl ether in a water bath at a temperature of about 35°C with the aid of a current of air: the residue melts \(<2.60\) between 128°C and 136°C.

Optical rotation \(<2.49\) [\(\alpha\)]\(D\): \(-13.5\) – \(-16.5\)° (after drying, 0.5 g, chloroform, 20 mL, 200 mm).

Purity (1) Clarity and color of solution—To 0.5 g of Levomepromazine Maleate add 10 mL of methanol, and dissolve by warming: the solution is clear, and colorless or pale yellow.

(2) Chloride \(<1.0\) Dissolve 0.5 g of Levomepromazine Maleate in 40 mL of methanol, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS until the color of the solution changes from red-purple through blue-purple to blue (indicator: 5 drops of bromocresol green-methylrosaniline chloride TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 36.14 mg of \(\text{C}_{19}\text{H}_{24}\text{N}_{2}\text{OS}\cdot\text{C}_{4}\text{H}_{4}\text{O}_{4}\)

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Levothyroxine Sodium Hydrate

レボチロキシンナトリウム水和物

![Chemical structure of Levothyroxine Sodium Hydrate](image)

\(\text{C}_{15}\text{H}_{10}\text{I}_{4}\text{NNaO}_{4}\cdot\text{xH}_{2}\text{O}\)

Monosodium \(O\)-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosinate hydrate [25416-65-3]

Levothyroxine Sodium Hydrate contains not less than 97.0% of levothyroxine sodium (\(\text{C}_{15}\text{H}_{10}\text{I}_{4}\text{NNaO}_{4}\cdot\text{xH}_{2}\text{O}\) 798.85), calculated on the dried basis.

Description Levothyroxine Sodium Hydrate occurs as a pale yellowish white to light yellow-brown powder. It is odorless.

It is slightly soluble in ethanol (95), and practically insolu-
ble in water and in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification (1) Heat 0.1 g of Levothyroxine Sodium Hydrate over a flame: a purple gas evolves.

(2) To 0.5 mg of Levothyroxine Sodium Hydrate add 8 mL of a mixture of water, ethanol (95), hydrochloric acid and sodium hydroxide TS (6:5:2:2), warm in a water bath for 2 minutes, cool, and add 0.1 mL of sodium nitrite TS. Allow to stand in a dark place for 20 minutes, and add 1.5 mL of ammonia solution (28): a yellowish red color is produced.

(3) Determine the absorption spectrum of a solution of Levothyroxine Sodium Hydrate in dilute sodium hydroxide TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Moistened Levothyroxine Sodium Hydrate with sulfuric acid, and ignite: the residue responds to the Qualitative Tests <1.069> (1) and (2) for sodium salt.

Optical rotation <2.49> [α]D = −5 to −6° (0.3 g, calculated on the dried basis, a mixture of ethanol (95) and sodium hydroxide TS (2:1), 10 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 0.3 g of Levothyroxine Sodium Hydrate in 10 mL of a mixture of ethanol (95) and sodium hydroxide TS (2:1) by warming: the solution is clear and pale yellow to pale yellow-brown in color.

(2) Soluble halides—Dissolve 0.01 g of Levothyroxine Sodium Hydrate in 10 mL of water and 1 drop of dilute nitric acid, shake, and filter. To the filtrate add water to make 10 mL, then add 3 drops of silver nitrate TS, and mix: the solution has no more opalescence than the following control solution.

Control solution: To 0.20 mL of 0.01 mol/L hydrochloric acid VS add 10 mL of water and 1 drop of dilute nitric acid, and proceed as directed above.

(3) Related substances—Dissolve 20 mg of Levothyroxine Sodium Hydrate in 2 mL of a mixture of ethanol (95) and ammonia solution (28) (14:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of ethanol (95) and ammonia solution (28) (14:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of t-butanol, t-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of ethanol (95), hydrochloric acid and sodium hydroxide TS (6:5:2:2), warm in a water bath for 2 minutes, cool, and filter. To the filtrate add 0.1 mL of sodium nitrite TS, and allow to stand in a dark place for 20 minutes. Add 1.5 mL of ammonia solution (28): a yellowish red color develops.

Loss on drying <2.47> 7–11% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Assay Weigh accurately about 25 mg of Levothyroxine Sodium Hydrate, and proceed as directed under Oxygen Flask Combustion Method <1.067>, using a mixture of 10 mL of sodium hydroxide solution (1 in 100) and 1 mL of a freshly prepared sodium bisulfate solution (1 in 100) as the absorbing liquid, and prepare the test solution. Apply a small amount of water to the upper part of apparatus A, pull out C carefully, and wash C, B and the inner wall of A with 40 mL of water. To the test solution add 1 mL of bromine-acetic acid TS, insert the stopper C, and shake vigorously for 1 minute. Remove the stopper, rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water, and add 0.5 mL of formic acid. Stopper the flask with C, and shake vigorously for 1 minute again. Remove the stopper, and rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water. Bubble the solution with enough nitrogen gas in the flask to remove the oxygen and excess bromine, add 0.5 g of potassium iodide to the solution, and dissolve. Add immediately 3 mL of dilute sulfuric acid, mix, and allow to stand for 2 minutes. Titrate <2.50> the solution with 0.02 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L sodium thiosulfate VS = 0.6657 mg of C15H10I4NNaO4

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Levothyroxine Sodium Tablets

レボチロキシンナトリウム錠

Levothyroxine Sodium Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of levothyroxine sodium (C15H10I4NNaO4: 798.85).

Method of preparation Prepare as directed under Tablets, with Levothyroxine Sodium Hydrate.

Identification (1) Weigh a quantity of powdered Levothyroxine Sodium Tablets, equivalent to 0.5 mg of Levothyroxine Sodium Hydrate according to the labeled amount, add 8 mL of a mixture of water, ethanol (95), hydrochloric acid and sodium hydroxide TS (6:5:2:2), warm in a water bath for 2 minutes, cool, and filter. To the filtrate add 0.1 mL of sodium nitrite TS, and allow to stand in a dark place for 20 minutes. Add 1.5 mL of ammonia solution (28): a yellowish red color develops.

(2) To a quantity of powdered Levothyroxine Sodium Tablets, equivalent to 1 mg of Levothyroxine Sodium Hydrate according to the labeled amount, add 10 mL of ethanol (95), shake, filter, and use the filtrate as the sample solution. Dissolve 0.01 g of levothyroxine sodium for thin-layer chromatography in 100 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of t-butanol, t-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray a solution of 0.3 g of ninhydrin in 100 mL of a
mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and heat at 100°C for 3 minutes: the spots obtained from the sample solution and the standard solution show a red-purple color, and has the same Rf value.

**Purity** Soluble halides—Weigh a quantity of powdered Levothyroxine Sodium Tablets, equivalent to 2.5 mg of Levothyroxine Sodium Hydrate according to the labeled amount, add 25 mL of water, warm to 40°C, shake for 5 minutes, and then boil for 5 minutes, add 3 drops of dilute nitric acid, and filter. To the filtrate add 3 drops of silver nitrate TS, and mix: the solution has no more opalescence than the following control solution.

Control solution: To 0.25 mL of 0.01 mol/L hydrochloric acid VS add 25 mL of water and 3 drops of dilute nitric acid, and proceed as directed above.

**Uniformity of dosage units** \( <6.02 \) Perform the test according to the following method: it meets the requirements of the Content uniformity test.

Place 1 tablet of Levothyroxine Sodium Tablets in a glass-stoppered centrifuge tube, and add exactly 10 mL of 0.01 mol/L sodium hydroxide TS, warm at 50°C for 15 minutes, and shake vigorously for 20 minutes. Centrifuge this solution, take 5 mL of the supernatant liquid, add exactly 1 mL of the internal standard solution, and use this solution as the sample solution. Perform the test with 20 \( \mu L \) of the sample solution as directed under Liquid Chromatography \( <2.01 \) according to the following conditions, and calculate the ratio of the peak area of levothyroxine to that of the internal standard. Calculate the mean value from the ratios of each peak area of 10 samples: the deviation (\( \% \)) of the mean value and the ratio of each peak area should be not more than 15%. When the deviation (\( \% \)) is more than 15%, and 1 sample shows not more than 25%, perform another test with 20 samples. Calculate the deviation (\( \% \)) of the mean value of the 30 samples used in the 2 tests and the ratio of each peak area: there should be not more than 1 sample with the deviation more than 15% but not more than 25%, and no sample should deviate by more than 25%.

**Internal standard solution**—A solution of ethinylestradiol in a mixture of acetonitrile and dilute phosphoric acid (1 in 10) (9:1) (3 in 40,000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: a constant wavelength between 220 nm and 230 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 10 to 25 cm in length, packed with octadecylsilanized silica gel.

Column temperature: A constant temperature at about 25°C.

Mobile phase: A mixture of methanol, water and phosphoric acid (1340:660:1).

Flow rate: Adjust the flow rate so that the retention time of levothyroxine is about 9 minutes.

Selection of column: To 5 mL of a solution of levothyroxine sodium in 0.01 mol/L sodium hydroxide TS (1 in 200,000) add 1 mL of the internal standard solution. Proceed with 20 \( \mu L \) of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of levothyroxine and the internal standard in this order with the resolution between these peaks being not less than 2.0.

**Lidocaine**

リドカイン

\[ \text{C}_{14}H_{22}N_{2}O: \text{234.34} \]

2-Diethylamino-N-(2,6-dimethylphenyl)acetamide \[ [137-58-6] \]

Lidocaine, when dried, contains not less than 99.0% of \( \text{C}_{14}H_{22}N_{2}O \).

**Description** Lidocaine occurs as white to pale yellow crystals or crystalline powder.

It is very soluble in methanol and in ethanol (95%), soluble in acetic acid (100) and in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

**Identification** (1) Dissolve 40 mg of Lidocaine in 10 mL of 1 mol/L hydrochloric acid TS, and add water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry \( <2.24 \),
and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Lidocaine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Lidocaine in 2 mL of dilute hydrochloric acid, and add water to make 10 mL: the solution is clear and colorless to light yellow.

(2) Chloride <1.03>—Dissolve 0.6 g of Lidocaine in 6 mL of dilute nitric acid, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.041%).

(3) Sulfate <1.14>—Dissolve 0.5 g of Lidocaine in 5 mL of dilute hydrochloric acid, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS, 5 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.096%).

(4) Heavy metals <1.07>—Carbonize 2.0 g of Lidocaine by gentle ignition. After cooling, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and fire the ethanol to burn. After cooling, add 10 mL of sulfuric acid, proceed according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Related substances—Dissolve 0.10 g of Lidocaine in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.63>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 2-propanol, water and formic acid (5:3:1:1) to a distance of about 10 cm, air-dry the plate, and dry more at 80 °C for 30 minutes. After cooling, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying <2.41>** Not more than 0.5% (1 g, in vacuum, silica gel, 24 hours).

**Residue on ignition <2.44>** Not more than 0.1% (1 g).

**Assay** Dissolve about 0.5 g of Lidocaine, previously dried and accurately weighed, in 20 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 1 drop of crystal violet TS) until the color of the solution changes from purple to blue-green through blue. Perform a blank determination, and make any necessary correction.

\[
\text{Each mL of 0.1 mol/L perchloric acid VS} = 23.43 \text{ mg of } \text{C}_{14}\text{H}_{22}\text{N}_2\text{O}\text{.}
\]

**Containers and storage** Containers—Tight containers.

**Method of preparation** Prepare as directed under Injections, with Lidocaine and an equivalent amount of Hydrochloric Acid.

No preservative is added in the case of intravenous injections.

**Description** Lidocaine Injection is a colorless, clear liquid. pH: 5.0 – 7.0

**Identification** To a volume of Lidocaine Injection, equivalent to 20 mg of lidocaine hydrochloride (C\(_{14}\)H\(_{22}\)N\(_2\)O.HCl) according to the labeled amount, add 1 mL of sodium hydroxide TS, and extract with 20 mL of hexane. To 10 mL of the hexane extract add 20 mL of 1 mol/L hydrochloric acid TS, and shake vigorously. Determine the absorption spectrum of the water layer as directed under Ultraviolet-visible Spectrophotometry <2.25>: it exhibits a maximum between 261 nm and 265 nm.

**Extractable volume <6.05>** It meets the requirement.

**Foreign insoluble matter <6.06>** Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter <6.07>** It meets the requirement.

**Sterility <4.06>** Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** To an exactly measured volume of Lidocaine Injection, equivalent to about 0.1 g of lidocaine hydrochloride (C\(_{14}\)H\(_{22}\)N\(_2\)O.HCl), add exactly 10 mL of the internal standard solution and 0.001 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 85 mg of lidocaine for assay, previously dried in a desiccator (in vacuum, silica gel) for 24 hours, dissolve in 0.5 mL of 1 mol/L hydrochloric acid TS and a suitable volume of 0.001 mol/L hydrochloric acid TS, and add exactly 10 mL of the internal standard solution, then add 0.001 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q\(_T\) and Q\(_S\), of the peak area of lidocaine to that of the internal standard.

\[
Q_T = \frac{M_S \times Q_S}{Q_T} \times 1.156
\]

Amount (mg) of lidocaine hydrochloride
(C\(_{14}\)H\(_{22}\)N\(_2\)O.HCl) = M\(_S\) \times Q\(_T\) / Q\(_S\) \times 1.156

M\(_S\): Amount (mg) of lidocaine for assay
**Limaprost Alfadex**

リマプロスト アルファデクス

\[
\text{C}_{22}\text{H}_{36}\text{O}_{5}\cdot x\text{C}_{6}\text{H}_{12}\text{O}_{6}
\]

(2E)-7-[(1R,2R,3R)-3-Hydroxy-2-[(1E,3S,5S)-3-hydroxy-5-methylnon-1-en-1-yl]-5-oxocyclopentyl]hept-2-enoic acid-α-cyclodextrin

[100459-01-6, limaprost:alfadex = 1:1; clathrate compound]

Limaprost Alfadex is a α-cyclodextrin clathrate compound of limaprost. It contains not less than 2.8% and not more than 3.2% of limaprost (C\text{_{22}}H\text{_{36}}O\text{_{5}}: 380.52), calculated on the anhydrous basis.

**Description** Limaprost Alfadex occurs as a white powder. It is freely soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in ethyl acetate.

It is hygroscopic.

**Identification (1)** Dissolve 20 mg of Limaprost Alfadex in 5 mL of water, add 5 mL of ethyl acetate, shake, centrifuge, and use the upper layer as the sample solution (1). Separately, to 20 mg of Limaprost Alfadex add 5 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid as the sample solution (2). Evaporate the solvent of the sample solutions (1) and (2) under reduced pressure, add 2 mL of sulfuric acid to each of the residue, and shake them for 5 minutes: the solution obtained from the sample solution (1) develops an orange-yellow color while the solution from the sample solution (2) does not develop any color.

(2) Dissolve 20 mg of Limaprost Alfadex in 5 mL of water, add 5 mL of ethyl acetate, shake, centrifuge, and evaporate the solvent of the upper layer under reduced pressure. Dissolve the residue in 2 mL of ethanol (95), 5 mL of 1,3-dinitrobenzene TS, add 5 mL of a solution of potassium hydroxide in ethanol (95) (17 in 100) while ice-cooling, and allow to stand in a dark place while ice-cooling for 20 minutes: a purple color develops.

(3) To 50 mg of Limaprost Alfadex add 1 mL of iodine TS, dissolve by heating in a water bath, and allow to stand: a dark blue precipitate is formed. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Optical rotation** <2.49° [α]D: +125 – 135° (0.1 g, calculated on the anhydrous basis, dilute ethanol, 20 mL, 100 mm).

**Purity** Related substances—Perform the test immediately after preparation of the sample solution. Dissolve 0.10 g of Limaprost Alfadex in 2 mL of water, add 1 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dilute ethanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 3 mL of the standard solution (1), add dilute ethanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 3 µL each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography (2.20) according to the following operating conditions, and determine each peak area by the automatic integration method: the area of the peak of 17-epi-isomer, having the relative retention time of the peak area of limaprost from the samples solution is not larger than 1/3 times the peak area of limaprost from the standard solution (2). The total area of the peaks other than limaprost from the samples solution is not larger than the peak area of limaprost from the standard solution (1).

**Operating conditions—**

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of limaprost beginning after the solvent peak.

**System suitability—**

Test for required detectability: To exactly 1 mL of the standard solution (1) add dilute ethanol to make exactly 10 mL. Confirm that the peak area of limaprost obtained from
Lincomycin Hydrochloride Hydrate

リンコマイシン塩酸塩水和物

C₁₈H₃₄N₂O₆S.HCl.H₂O: 461.01
Methyl 6,8-dideoxy-6-[(2S,4R)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-ᴅ-erythro-α-D-galacto-octopyranoside monohydrochloride monohydrate [7179-49-9]

Lincomycin Hydrochloride Hydrate is the hydrochloride of a substance having antibacterial activity produced by the growth of *Streptomyces lincolnensis* var. *lincolnensis*.

It contains not less than 825 μg (potency) per mg, calculated on the anhydrous basis. The potency of Lincomycin Hydrochloride Hydrate is expressed as mass (potency) of lincomycin (C₁₈H₃₄N₂O₆S: 406.54).

**Description**

Lincomycin Hydrochloride Hydrate occurs as white, crystals or crystalline powder.

It is freely soluble in water and in methanol, sparingly soluble in ethanol (95), and very slightly soluble in acetone.

**Identification**

1. Determine the infrared absorption spectrum of Lincomycin Hydrochloride Hydrate as directed in the paste method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum or the spectrum of Lincomycin Hydrochloride Hydrate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

2. A solution of Lincomycin Hydrochloride Hydrate (1 in 100) responds to the Qualitative Tests (1.09) (2) for chloride.

**Optical rotation** (2.49) [α]₁₄°: +135 – +150° (0.5 g, water, 25 mL, 100 mm).

**pH** (2.54) Dissolve 0.10 g of Lincomycin Hydrochloride Hydrate in 1 mL of water: 3.0 – 5.5.

**Purity**

1. Clarity and color of solution—Dissolve 1.0 g of Lincomycin Hydrochloride Hydrate in 10 mL of water: the solution is clear and colorless.

2. Heavy metals (1.07)—Proceed with 2.0 g of Lincomycin Hydrochloride Hydrate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 5 ppm).

3. Lincomycin B—Perform the test with 20 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the peak areas of lincomycin and lincomycin B, having the relative retention time of about 0.5 with respect to lincomycin, by the automatic integration method: the peak area of lincomycin B is not more than 5.0% of the sum of the peak areas of lincomycin and linco-
Lincomycin Hydrochloride Injection

リンコマイシン塩酸塩注射液

Lincomycin Hydrochloride Injection is an aqueous injection.

It contains not less than 93.0% and not more than 107.0% of the labeled potency of lincomycin (C18H34N2O6S: 406.54).

Method of preparation Prepare as directed under Injections, with Lincomycin Hydrochloride Hydrate.

Description Lincomycin Hydrochloride Injection is a clear, colorless liquid.

Identification To a volume of Lincomycin Hydrochloride Injection, equivalent to 30 mg (potency) of Lincomycin Hydrochloride Hydrate according to the labeled amount, add 30 mL of water, and use this solution as the sample solution. Separately, dissolve 10 mg (potency) of Lincomycin Hydrochloride RS in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Dissolve 150 g of ammonium acetate in 800 mL of water, adjust the pH to 9.6 with ammonia solution (28), and add water to make 1000 mL. To 80 mL of this solution add 40 mL of 2-propanol and 90 mL of ethyl acetate, shake, develop the plate with the upper layer of this solution to a distance of about 15 cm, and air-dry the plate. Spray evenly a solution of potassium permanganate (1 in 1000) on the plate: the principal spots from the sample solution and standard solution show the same Rf value.

pH (2.48) 3.5 – 5.5

Bacterial endotoxins (4.01) Less than 0.50 EU/mg (potency).

Extractable volume (6.05) It meets the requirement.

Foreign insoluble matter (6.06) Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter (6.07) It meets the requirement.

Sterility (4.06) Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Pipet a volume of Lincomycin Hydrochloride Injection, equivalent to about 0.3 g (potency) of Lincomycin Hydrochloride Hydrate, add the mobile phase to make exactly 20 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Lincomycin Hydrochloride RS, equivalent to 20 mg (potency), dissolve in the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Lincomycin Hydrochloride Hydrate.

Amount [mg (potency)] of lincomycin (C18H34N2O6S) = \( M_S \times A_r / A_s \times 15 \)

Container and storage Containers—Tight containers.
M₅: Amount [mg (potency)] of Lincomycin Hydrochloride RS

**Containers and storage** Containers—Hermetic containers.

**Liothyronine Sodium**

リオチロニンナトリウム

C₁₅H₁₁I₃N₄NaO₄: 672.96

Monosodium O-(4-hydroxy-3-iodophenyl)-3,5-diiodo-L-tyrosinate

[H-06-1]

Liothyronine Sodium contains not less than 95.0% of C₁₅H₁₁I₃N₄NaO₄, calculated on the dried basis.

**Description** Liothyronine Sodium occurs as a white to light brown powder. It is odorless.

It is slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It dissolves in sodium hydroxide TS and in ammonia TS.

**Identification** (1) To 5 mL of a solution of Liothyronine Sodium in ethanol (95) (1 in 1000) add 1 mL of ninhydrin TS, and warm in a water bath for 5 minutes: a purple color develops.

(2) Heat 0.02 g of Liothyronine Sodium with a few drops of sulfuric acid over a flame: a purple gas is evolved.

(3) Determine the absorption spectrum of a solution of Liothyronine Sodium in ethanol (95) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry [2.40], and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Ignite 0.02 g of Liothyronine Sodium until thoroughly charred. After cooling, add 5 mL of water to the residue, shake, and filter: the filtrate responds to the Qualitative Tests [1.09] (1) for sodium salt.

**Optical rotation** [2.49] [α]D: +18° – +22° (0.2 g, calculated on the dried basis, a mixture of ethanol (95) and 1 mol/L hydrochloric acid TS (4:1), 10 mL, 100 mm).

**Purity** (1) Soluble halide—To 10 mg of Liothyronine Sodium add 10 mL of water and 1 drop of dilute nitric acid, shake for 5 minutes, and filter. Add water to the filtrate to make 10 mL, and mix with 3 drops of silver nitrate TS: the solution shows no more turbidity than the following control solution.

Control solution: To 0.35 mL of 0.01 mol/L hydrochloric acid VS add 1 drop of dilute nitric acid and water to make 10 mL, and add 3 drops of silver nitrate TS.

(2) Iodine and iodide—Dissolve 0.10 g of Liothyronine Sodium in 10 mL of dilute sodium hydroxide TS and 15 mL of water, add 5 mL of dilute sulfuric acid, and allow to stand for 10 minutes with occasional shaking. Filter the mixture into a Nessler tube, add 10 mL of chloroform and 3 drops of a solution of potassium iodate (1 in 100) to the filtrate, mix for 30 seconds, and allow to stand: the chloroform layer has no more color than the following control solution.

Control solution: Weigh exactly 0.111 g of potassium iodide, and dissolve in water to make 1000 mL. Pipet 1 mL of this solution, add 10 mL of dilute hydroxide TS, 14 mL of water and 5 mL of dilute sulfuric acid, and mix. Filter the mixture into a Nessler tube, and perform the test with the filtrate in the same manner as for the sample.

(3) Related substances—Dissolve 0.15 g of Liothyronine Sodium in 5 mL of dilute ammonia TS (1 in 3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dilute ammonia TS (1 in 3) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography [2.67]. Spot 1 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-butanol, t-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and dry the plate at 100°C for 3 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** [2.41] Not more than 4.0% (0.2 g, 105°C, 2 hours).

**Assay** Weigh accurately about 25 mg of Liothyronine Sodium, and proceed as directed under Oxygen Flask Combustion Method [1.06], using a mixture of 10 mL of a solution of sodium hydroxide (1 in 100) and 1 mL of a freshly prepared solution of sodium bisulfate (1 in 100) as the absorbing liquid, and prepare the test solution. Apply a small amount of water to the upper part of apparatus A, pull out C carefully, and wash C, B and the inner wall of A with 40 mL of water. To the test solution add 1 mL of bromine-acetic acid TS, insert the stopper C, and shake vigorously for 1 minute. Remove the stopper, rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water, and add 0.5 mL of formic acid. Stopper the flask with C, and shake vigorously for 1 minute again. Remove the stopper, and rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water again. Bubble the solution with enough nitrogen gas in the flask to remove the oxygen and excess bromine, add 0.5 g of potassium iodide to the solution, and dissolve. Add immediately 3 mL of dilute sulfuric acid, mix, and allow to stand for 2 minutes. Titrate [2.50] the solution with 0.02 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L sodium thiosulfate VS = 0.7477 mg of C₁₅H₁₁I₃N₄NaO₄

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.
Liothyronine Sodium Tablets

リオチロニンナトリウム錠

Liothyronine Sodium Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of liothyronine sodium (C₁₅H₁₁I₃NNaO₄: 672.96).

Method of preparation Prepare as directed under Tablets, with Liothyronine Sodium.

Identification (1) To a glass-stoppered centrifuge tube add a portion of finely powdered Liothyronine Sodium Tablets, equivalent to 0.1 mg of Liothyronine Sodium according to the labeled amount, add 30 mL of dilute sodium hydroxide TS, shake vigorously, and centrifuge. Transfer the supernatant liquid to a separator, add 10 mL of dilute hydrochloric acid, and extract with two 20-mL portions of ethyl acetate. Filter each extract successively through absorbent cotton previously overlaid with 8 g of anhydrous sodium sulfate. Evaporate the filtrate on a water bath to dryness with the aid of a current of nitrogen. Dissolve the residue in 0.5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 10 mg of liothyronine sodium for thin-layer chromatography in methanol to make 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (<2.0>). Spot 20 μL of each sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of t-butanol, t-amy alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and dry the plate at 100°C for 3 minutes. The spots obtained from the sample solution and the standard solution show a red-purple color, and has the same Rf value.

(2) The colored solution obtained in the Assay is blue in color.

Uniformity of dosage units (<6.02) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Place 1 tablet of Liothyronine Sodium Tablets in a glass-stoppered centrifuge tube, add exactly 10 mL of 0.01 mol/L sodium hydroxide TS, warm at 50°C for 15 minutes, and shake vigorously for 20 minutes. Centrifuge for 5 minutes, and filter the supernatant liquid, if necessary. Pipet a definite volume of this solution, and add a volume of 0.01 mol/L sodium hydroxide TS to prepare a definite volume of a solution containing about 0.5 μg of liothyronine sodium (C₁₅H₁₁I₃NNaO₄) per mL. Pipet 5 mL of this solution, add exactly 1 mL of the internal standard solution, and use this solution as the sample solution. Perform the test with 200 μL of the sample solution as directed under Liquid Chromatography (<2.0>) according to the following conditions, and calculate the ratio of the peak area of the liothyronine to that of the internal standard. Calculate the mean value of the ratios of each peak area of 10 samples: the deviation (%) of each ratio of the peak area from the mean value should be not more than 15%. When the deviation (%) is more than 15%, 1 sample shows not more than 25%, perform another test with 20 samples. Calculate the deviation (%) of each ratio of the peak area from the mean value of the 30 samples used in the two tests: there should be not more than 1 sample with the deviation more than 15% but not more than 25%, and no sample should deviate by more than 25%.

Internal standard solution—A solution of propylparahydroxybenzoate in a mixture of methanol and diluted phosphoric acid (1 in 10) (9:1) (1 in 250,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylslylanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Diluted methanol (57 in 100).

Flow rate: Adjust the flow rate so that the retention time of liothyronine is about 9 minutes.

System suitability—

System performance: To 5 mL of a solution of liothyronine sodium in 0.01 mol/L sodium hydroxide TS (1 in 2,000,000) add 1 mL of the internal standard solution, and use this solution as the solution for system suitability test. When the procedure is run with 200 μL of this solution under the above operating conditions, the internal standard and liothyronine are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 200 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the ratios of the peak area of liothyronine to that of the internal standard is not more than 1.0%.

Assay Weigh accurately not less than 20 Liothyronine Sodium Tablets, and finely powder. Place an accurately weighed portion of the powder, equivalent to about 50 μg of liothyronine sodium (C₁₅H₁₁I₃NNaO₄), in an agate mortar, add 1 g of powdered potassium carbonate, and mix well. Transfer the mixture cautiously to a porcelain crucible, and compact the contents by gently tapping the crucible on a table. Add an additional 1.5 g of powdered potassium carbonate to the same agate mortar, mix well with any content adhering to the mortar, cautiously overlay the mixture on the top of the same porcelain crucible, and compact the charge again in the same manner. Ignite the combined mixture in the crucible between 675°C and 700°C for 30 minutes. Cool, add a few mL of water to the crucible, heat gently to boiling, and filter the contents of the crucible through a glass filter (G4) into a 20-mL volumetric flask. Wash the residue with water, and combine the washings with the filtrate. Cool, add water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 75 mg of potassium iodide for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 200 mL. Measure exactly 5 mL of the solution, and add a solution of potassium carbonate (1 in 8) to make exactly 100 mL. To 2 mL of this solution, exactly measured, add a solution of potassium carbonate (1 in 8) to make exactly 20 mL, and use the solution as the standard solution. Pipet 5 mL each of the sample solution and the standard solution into
glass-stoppered test tubes, add 3.0 mL of diluted sulfuric acid (4 in 25) and 2.0 mL of potassium permanganate TS, and heat on a water bath for 15 minutes. Cool, add 1.0 mL of diluted sodium nitrite TS (1 in 10), swirl to mix, and add 1.0 mL of a solution of ammonium amidosulfate (1 in 10). Allow to stand at room temperature for 10 minutes with occasional shaking. Then add 1.0 mL of potato starch TS and 1.0 mL of a freshly prepared, diluted potassium iodide TS (1 in 40), swirl to mix, and transfer each solution to a 20-mL volumetric flask. Rinse the test tube with water, collect the washings in the volumetric flask, add water to make 20 mL, and allow to stand for 10 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 5 mL of potassium carbonate (1 in 8) in the same manner as the sample solution as the blank. Determine the absorbances, \( A_1 \) and \( A_3 \), of the subsequent solutions of the sample solution and the standard solution at the wavelength of maximum absorption at about 600 nm, respectively.

\[
\text{Amount (mg) of liothyronine sodium } (C_{13}H_{11}I_3NNaO_4) = M_s \times A_1/A_3 \times 1/2000 \times 1.351
\]

\( M_s \): Amount (mg) of potassium iodide for assay

**Containers and storage** Containers—Tight containers.

**Storage**—Light-resistant.

**Lisinopril Hydrate**

リシノプリル水和物

![Image](https://example.com/image)

\( C_{21}H_{31}N_3O_5 \cdot 2H_2O \): 441.52


Lisinopril Hydrate contains not less than 98.5\% and not more than 101.0\% of lisinopril (C\(_{21}\)H\(_{31}\)N\(_3\)O\(_5\): 405.49), calculated on the anhydrous basis.

**Description** Lisinopril Hydrate occurs as a white crystalline powder, having a slight characteristic odor.

It is soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (99.5).

Melting point: about 160°C (with decomposition).

**Identification** (1) Determine the absorption spectrum of a solution of Lisinopril Hydrate in methanol (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Lisinopril Hydrate as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** \((\alpha)_D^20 \approx -43.0 \sim -47.0^\circ\) (0.25 g calculated on the anhydrous basis, 0.25 mL/L zinc acetate buffer solution, pH 6.4, 25 mL, 100 nm).

**Purity (1)** Heavy metals \(<1.07\%\)—Proceed with 2.0 g of Lisinopril Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve about 0.10 g of Lisinopril Hydrate in 50 mL of water, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 15 \(\mu\)L of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 1.2 with respect to lisinopril, is not larger than 1/5 times the peak area of lisinopril from the standard solution, the area of the peak other than lisinopril and the peak mentioned above is not larger than 2/15 times the peak area of lisinopril from the standard solution, and the total area of the peaks other than lisinopril is not larger than the peak area of lisinopril from the standard solution.

**Operating conditions**—


Column: A stainless steel column 4.0 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase A: Diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2).

Mobile phase B: A mixture of diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2) and acetonitrile for liquid chromatography (3:2).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10</td>
<td>90 (\rightarrow) 50</td>
<td>10 (\rightarrow) 50</td>
</tr>
<tr>
<td>10 – 25</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Flow rate: About 1.5 mL per minute.

Time span of measurement: About 2.5 times as long as the retention time of lisinopril beginning after the solvent peak.

**System suitability**—

Test for required detectability: Measure exactly 2.5 mL of the standard solution, and add water to make exactly 50 mL. Confirm that the peak area of lisinopril obtained with 15 \(\mu\)L of this solution is equivalent to 3.5 to 6.5\% of that with 15 \(\mu\)L of the standard solution.

System performance: To 10 mg of Lisinopril Hydrate and 2 mL of a solution of anhydrous caffeine (1 in 1000) add water to make 200 mL. When the procedure is run with 15
Lisinopril Tablets

Lisinopril Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of lisinopril (C₂₁H₃₁N₃O₅): 405.49.

**Method of preparation**Prepare as directed under Tablets, with Lisinopril Hydrate.

**Identification**To an amount of powdered Lisinopril Tablets, equivalent to 10 mg of lisinopril (C₂₁H₃₁N₃O₅), add 10 mL of methanol, shake for 20 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of lisinopril in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography.<sup>2.46</sup> Spot 30 μL of each sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetonitrile, acetic acid (100), water and ethyl acetate (2:2:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 120°C: the principal spot with the sample solution and the spot with the standard solution show a red-purple color and their Rₙ values are the same.

**Purity**Related substances—Powder not less than 20 Lisinopril Tablets. Take a portion of the powder, equivalent to about 25 mg of lisinopril (C₂₁H₃₁N₃O₅), add 25 mL of water, shake for 20 minutes, filter, and use the filtrate as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography<sup>2.01</sup> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of lisinopril diketopiperazine, having the relative retention time of about 2.0 with respect to lisinopril, is not larger than 2/3 times the peak area of lisinopril from the standard solution.

**Operating conditions**—
Proceed as directed in the operating conditions in the Purity (2) under Lisinopril Hydrate.

**System suitability**—
Test for required detectability: To exactly 2.5 mL of the standard solution add water to make exactly 50 mL. Confirm that the peak area of lisinopril obtained with 15 μL of this solution is equivalent to 3.5 to 6.5% of that with 15 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Purity (2) under Lisinopril Hydrate.

**Uniformity of dosage units**<sup>6.02</sup> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Lisinopril Tablets add exactly 5 mL each of the internal standard solution per every 1 mg of lisinopril (C₂₁H₃₁N₃O₅), shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Hereafter, proceed as directed in the Assay.

\[
\text{Amount (mg) of lisinopril (C}_2\text{H}_3\text{N}_2\text{O}_3) = M_S \times \frac{Q}{V} \times \frac{1}{10} \times C
\]

\[
M_S: \text{Amount (mg) of lisinopril for assay, calculated on the anhydrous basis}
\]

\[
C: \text{Labeled amount (mg) of lisinopril (C}_2\text{H}_3\text{N}_2\text{O}_3) \text{ in 1 tablet}
\]

**Internal standard solution**—A solution of anhydrous caffeine (1 in 20,000).

**Dissolution**<sup>6.10</sup> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate of a 5 mg tablet in 60 minutes and that of a 10-mg tablet in 90 minutes is not less than 80%, and that of a 20-mg tablet in 90 minutes is not less than 75%.

Start the test with 1 tablet of Lisinopril Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 5.6 μg of lisinopril (C₂₁H₃₁N₃O₅) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of lisinopril for assay, separately determined the water<sup>2.46</sup> in the same manner as Lisinopril Hydrate, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography<sup>2.01</sup> according to the following conditions, and determine the peak areas, A₁ and A₃, of lisinopril.

Dissolution rate (%) with respect to the labeled amount of lisinopril (C₂₁H₃₁N₃O₅) = \[ M_S \times \frac{A_1}{A_3} \times \frac{V}{V'} \times \frac{1}{C} \times 36 \]

\[
M_S: \text{Amount (mg) of lisinopril for assay, calculated on the}
\]

μL of this solution under the above operating conditions, lisinopril and caffeine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 15 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lisinopril is not more than 2.0%.

**Water**<sup>2.40</sup> Not less than 8.0% and not more than 9.5% (0.3 g, volumetric titration, back titration).

**Residue on ignition**<sup>2.46</sup> Not more than 0.1% (1 g).

**Assay**Weigh accurately about 0.66 g of Lisinopril Hydrate, dissolve in 80 mL of water, and titrate<sup>2.50</sup> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 40.55 mg of C₂₁H₃₁N₃O₅

**Containers and storage** Containers—Well-closed containers.
Lithium Carbonate

**JP XVI**

Lithium Carbonate, when dried, contains not less than 99.5% of Li₂CO₃.

**Description** Lithium Carbonate occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in water, slightly soluble in hot water, and practically insoluble in ethanol (95) and in diethyl ether.

It dissolves in dilute acetic acid.

The pH of a solution dissolved 1.0 g of Lithium Carbonate in 100 mL or water is between 10.9 and 11.5.

**Identification** (1) Perform the test as directed under Flame Coloration Test <1.045> (1) with Lithium Carbonate: a persistent red color appears.

(2) Dissolve 0.2 g of Lithium Carbonate in 3 mL of dilute hydrochloric acid, and add 4 mL of sodium hydroxide TS and 2 mL of disodium hydrogen phosphate TS: a white precipitate is produced. To the precipitate add 2 mL of dilute hydrochloric acid: it dissolves.

(3) A solution of Lithium Carbonate (1 in 100) responds to the Qualitative Tests <1.09> for carbonate.

**Purity** (1) Clarity and color of solution—Dissolve 0.10 g of Lithium Carbonate in 10 mL of water by warming: the solution is clear and colorless.

(2) Acetic acid-insoluble substances—Take 1.0 g of Lithium Carbonate, dissolve in 40 mL of dilute acetic acid, filter the insoluble substances using filter paper for quantitative analysis, wash with five 10-mL portions of water, and ignite the insoluble substances together with the filter paper to incinerate: the mass of the residue is not more than 1.5 mg.

(3) Chloride <1.03>—To 0.40 g of Lithium Carbonate add 10 mL of water and 7 mL of dilute nitric acid, and dissolve by heating to boil. After cooling, add 6 mL of dilute nitric acid, and dilute with water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.022%).

(4) Sulfate <1.14>—To 0.40 g of Lithium Carbonate add 10 mL of water and 4 mL of dilute hydrochloric acid, and dissolve by heating to boil. After cooling, add 1 mL of dilute hydrochloric acid, and dilute with water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).
(5) Heavy metals \(<1.0\,\text{ppm}>\)—To 4.0 g of Lithium Carbonate add 5 mL of water, gradually add 10 mL of hydrochloric acid while mixing, and dissolve. Evaporate the solution on a water bath to dryness. To the residue add 10 mL of water, and dissolve. Place the solution in a Nessler tube, add 1 drop of phenolphthalein TS, add ammonia TS until the solution shows a pale red color, then add 2.0 mL of Standard Lead Bath to dryness. To the residue add 49.5 mg of magnesium sulfate heptahydrate, previously dried at 105°C for 2 hours and heated at 450°C for 3 hours, in water to make 1000 mL. To this solution add 3 mL of solution B obtained in (7), 0.2 mL of a solution of titanium yellow (1 in 1000) and water to make 20 mL, and proceed in the same manner.

(6) Iron \(<1.0\,\text{ppm}>\)—Prepare the test solution with 1.0 g of Lithium Carbonate, according to Method B. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Aluminum—To 5.0 g of Lithium Carbonate add 20 mL of water, add gradually 15 mL of hydrochloric acid while stirring, and evaporate to dryness on a water bath. To the residue add 50 mL of water to dissolve, filter if necessary, and assign this solution as solution A. Separately, evaporate 15 mL of hydrochloric acid to dryness on a water bath, then proceed in the same manner, and assign the solution so obtained as solution B. To 10 mL of solution A add 10 mL of water and 5 mL of acetic acid-sodium acetate buffer solution, pH 4.5, and shake. Add 1 mL of a solution of \(L\)-ascorbic acid (1 in 100), 2 mL of ammonium TS and water to make 50 mL, shake well, and allow to stand for 10 minutes: the solution has no more color than the following control solution.

Control solution: Dissolve 0.1758 g of aluminum potassium sulfate dodecahydrate in water to make 1000 mL. To 1.0 mL of this solution add 10 mL of solution B obtained in (7) and water to make 20 mL, add 5 mL of acetic acid-sodium acetate buffer solution, pH 4.5, and proceed in the same manner.

(8) Barium—To 20 mL of solution A obtained in (7) add 6 mL of water, 0.5 mL of dilute hydrochloric acid, 3 mL of ethanol (95) and 2 mL of potassium sulfate TS, and allow to stand for 1 hour: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 17.8 mg of barium chloride dihydrate in water to make 1000 mL. To 6 mL of this solution add 20 mL of solution B obtained in (7), 0.5 mL of dilute hydrochloric acid and 3 mL of ethanol (95), and proceed in the same manner.

(9) Calcium—Weigh accurately about 5 g of Lithium Carbonate, add 50 mL of water and 15 mL of hydrochloric acid, and dissolve. Remove carbon dioxide from the solution by boiling, add 5 mL of ammonium oxalate TS, then make alkaline with ammonia TS, and allow to stand for 4 hours. Filter the produced precipitate through a glass filter (G4), wash with warm water until the turbidity of the washing is not produced with calcium chloride TS within 1 minute. Transfer the precipitate and the glass filter into a beaker, add water until the glass filter is covered with water, then add 3 mL of sulfuric acid, heat between 70°C and 80°C, and titrate with 0.02 mol/L potassium permanganate VS until a pale red color persists for 30 seconds: the amount of calcium (\(\text{Ca}: 40.08\)) is not more than 0.05%.

Each mL of 0.02 mol/L potassium permanganate VS = 2.004 mg of Ca

(10) Magnesium—To 3.0 mL of solution A obtained in (7) add 0.2 mL of a solution of titan yellow (1 in 1000) and water to make 20 mL, then add 5 mL of sodium hydroxide (3 in 20), and allow to stand for 10 minutes: the solution has no more color than the following control solution.

Control solution: Dissolve 49.5 mg of magnesium sulfate heptahydrate, previously dried at 105°C for 2 hours and heated at 450°C for 3 hours, in water to make 1000 mL. To this solution add 3 mL of solution B obtained in (7), 0.2 mL of a solution of titanium yellow (1 in 1000) and water to make 20 mL, and proceed in the same manner.

(11) Potassium—Dissolve 1.0 g of Lithium Carbonate in water to make 100 mL, and use this solution as the sample solution. To 5 mL of the sample solution add 1.0 mL of dilute acetic acid, shake, add 5 mL of a solution of sodium tetr phenylborate (1 in 30), shake immediately, and allow to stand for 10 minutes: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 9.5 mg of potassium chloride in water to make 1000 mL. To 5 mL of this solution add 1.0 mL of dilute acetic acid, shake, and proceed in the same manner.

(12) Sodium—Weigh accurately about 0.8 g of Lithium Carbonate, dissolve in water to make exactly 100 mL, and use this solution as the sample stock solution. Measure exactly 25 mL of the sample stock solution, add water to make exactly 100 mL, and use this solution as the sample solution (1). Separately, weigh accurately 25.4 mg of sodium chloride, dissolve in water to make exactly 1000 mL, and use this solution as the standard solution. Measure exactly 25 mL of the sample stock solution, add exactly 20 mL of the standard solution, then add water to make exactly 100 mL, and use this solution as the sample solution (2). Determine emission intensities of sodium using a flame photometer with the sample solution (1) and the sample solution (2) under the following conditions. Adjust the wavelength dial to 589 nm, atomize the sample solution (2) into the flame, then adjust the sensitivity so that the emission intensity \(L_T\) shows 100 adjustment, and determine emission intensity \(L_T\) of the sample solution (1). Then, make the other conditions identical, change the wavelength dial to 580 nm, determine emission intensity \(L_S\) of the sample solution (1): the amount of sodium, calculated from the following equation, is not more than 0.05%.

\[
\text{Amount (mg of sodium)} = \frac{(L_T - L_0)}{(L_S - L_T)} \times M' / M \times 100
\]

\(M\): Amount (mg) of the sample in 25 mL of the sample stock solution

\(M'\): Amount (mg) of sodium in 20 mL of the standard solution.

(13) Arsenic \(<2.4\,\text{ppm}>\)—Prepare the test solution with 1.0 g of Lithium Carbonate, add 2 mL of water and 3 mL of hydrochloric acid, and perform the test (not more than 2 ppm).
Assay  Weigh accurately about 1 g of Lithium Carbonate, previously dried, add exactly 100 mL of water and 50 mL of 0.5 mol/L sulfuric acid VS, remove carbon dioxide by boiling gently, cool, and titrate $< 2.50 \Delta$ the excess sulfuric acid with 1 mol/L sodium hydroxide VS until the color of the solution changes from red to yellow (indicator: 3 drops of methyl red TS). Perform a blank determination.

Each mL of 0.5 mol/L sulfuric acid VS $= 36.95 \text{ mg of Li}_2\text{CO}_3$

Containers and storage  Containers—Well-closed containers.

Lorazepam  ロラゼパム

$\text{C}_{15}\text{H}_{10}\text{Cl}_2\text{N}_2\text{O}_2$: 321.16

(3RS)-7-Chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-2H-1,4-benzodiazepin-2-one [846-49-1]

Lorazepam, when dried, contains not less than 98.5% of $\text{C}_{15}\text{H}_{10}\text{Cl}_2\text{N}_2\text{O}_2$.

Description  Lorazepam occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in ethanol (95) and in acetone, slightly soluble in diethyl ether, and practically insoluble in water.

It is gradually colored by light.

Identification  (1)  To 0.02 g of Lorazepam add 15 mL of dilute hydrochloric acid, boil for 5 minutes, and cool: the solution responds to the Qualitative Tests $< 1.09$ for primary aromatic amines.

(2)  Determine the absorption spectrum of a solution of Lorazepam in ethanol (95) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry $< 2.24 \Delta$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3)  Determine the infrared absorption spectrum of Lorazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $< 2.25 \Delta$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4)  Perform the test with Lorazepam as directed under Flame Coloration Test $< 1.06 \Delta$: a green color appears.

Absorbance $< 2.24 \Delta$ $E_{\text{1cm}}^{1\text{cm}}$ (229 nm): 1080 – 1126 (after drying, 1 mg, ethanol (95), 200 mL).

Purity  (1)  Chloride $< 1.07 \Delta$—To 1.0 g of Lorazepam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2)  Heavy metals $< 1.07 \Delta$—Proceed with 1.0 g of Lorazepam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 0.02 ppm).

(3)  Arsenic $< 1.11 \Delta$—Prepare the test solution with 1.0 g of Lorazepam according to Method 3, and perform the test (not more than 2 ppm).

(4)  Related substances—Dissolve 0.10 g of Lorazepam in 20 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $< 2.03 \Delta$. Spot 10 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, 1,4-dioxane and acetic acid (100) (91:5:4) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying $< 2.41 \Delta$  Not more than 0.5% (1 g, in vacuum, 105°C, 3 hours).

Residue on ignition $< 2.44 \Delta$  Not more than 0.3% (1 g).

Assay  Weigh accurately about 0.4 g of Lorazepam, previously dried, dissolve in 50 mL of acetone, and titrate $< 2.50 \Delta$ with 0.1 mol/L tetrabutylammonium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L tetrabutylammonium hydroxide VS $= 32.12 \text{ mg of C}_{15}\text{H}_{10}\text{Cl}_2\text{N}_2\text{O}_2$

Containers and storage  Containers—Tight containers. Storage—Light-resistant.

Losartan Potassium  ロサルタンカリウム

$\text{C}_{22}\text{H}_{22}\text{ClKN}_6\text{O}: 461.00$

Monopotassium 5-[[4-(2-butyl-4-chloro-5-hydroxymethyl-1H-imidazol-1-yl)phenyl]-2-yl]-1H-tetrazol-1-ide [124750-59-8]

Losartan Potassium contains not less than 98.5% and not more than 101.0% of $\text{C}_{22}\text{H}_{22}\text{ClKN}_6\text{O}$, calcu-
luted on the anhydrous basis.

**Description** Losartan Potassium occurs as a white crystaline powder.

It is very soluble in water, and freely soluble in methanol and in ethanol (99.5).

**Identification (1)** Determine the absorption spectrum of a solution of Losartan Potassium in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Losartan Potassium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Losartan Potassium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Losartan Potassium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Losartan Potassium responds to the Qualitative Tests <1.00> (1) for potassium salt.

(4) Perform the test with Losartan Potassium as directed under Flame Coloration Test <1.045> (2): a green color appears.

**Purity (1)** Heavy metals <1.07>—Proceed with 2.0 g of Losartan Potassium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 30 mg of Losartan Potassium in 100 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peaks of solvent and losartan obtained from the sample solution is not larger than 1/10 times the peak area of losartan from the standard solution, and the total area of the peaks other than the peak of losartan from the sample solution is not larger than 3/10 times the peak area of losartan from the standard solution.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Diluted phosphoric acid (1 in 1000).

Mobile phase B: Acetonitrile.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 25</td>
<td>75 → 10</td>
<td>25 → 90</td>
</tr>
<tr>
<td>25 - 35</td>
<td>10</td>
<td>90</td>
</tr>
</tbody>
</table>

Flow rate: 1.0 mL per minute.

Time span of measurement: 35 minutes after injection of the sample.

**System suitability**—

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 10 mL. Confirm that the peak area of losartan obtained from 10 μL of this solution is equivalent to 7 to 13% of that of losartan from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of losartan are not less than 10,000 and not more than 1.3, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of losartan is not more than 2.0%.

(3) Residual solvent—Being specified separately.

**Water <2.46>** Not more than 0.5% (0.25 g, volumetric titration, direct titration).

**Assay** Weigh accurately about 25 mg each of Losartan Potassium and Losartan Potassium RS (separately, determine the water <2.46> in the same manner as Losartan Potassium), dissolve separately in methanol to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of losartan in each solution.

\[
\text{Amount (mg) of losartan potassium (C}_{22}\text{H}_{22}\text{ClKN}_{6}\text{O)} = M_S \times \frac{A_T}{A_S}
\]

\[
M_S: \text{Amount (mg) of Losartan Potassium RS, calculated on the anhydrous basis}
\]

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of losartan is about 6 minutes.

**System suitability**—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of losartan are not less than 5500 and not more than 1.4, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of losartan is not more than 1.0%.

Containers and storage  Containers—Tight containers.

**Loxoprofen Sodium Hydrate**

ロキソプロフェナートリウム水和物

\[
\text{C}_{15}\text{H}_{17}\text{NaO}_{3} \cdot 2\text{H}_{2}\text{O} : 304.31
\]

Monosodium 2-[4-[(2-oxocyclopentyl)methyl]phenyl]propanoate dihydrate [80382-23-6]

Loxoprofen Sodium Hydrate contains not less than 98.5% of loxoprofen sodium (\(\text{C}_{15}\text{H}_{17}\text{NaO}_{3} : 268.28\)), calculated on the anhydrous basis.

**Description**  Loxoprofen Sodium Hydrate occurs as white to yellowish white crystals or crystalline powder.

It is very soluble in water and in methanol, freely soluble in ethanol (95), and practically insoluble in diethyl ether.

A solution of Loxoprofen Sodium Hydrate (1 in 20) does not show optical rotation.

The pH of a solution of Loxoprofen Sodium Hydrate in freshly boiled and cooled water (1 in 20) is between 6.5 and 8.5.

**Identification** (1)  Determine the absorption spectrum of a solution of Loxoprofen Sodium Hydrate (1 in 55,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\rangle\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2)  Determine the infrared absorption spectrum of Loxoprofen Sodium Hydrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry \(<2.25\rangle\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3)  A solution of Loxoprofen Sodium Hydrate (1 in 10) responds to the Qualitative Tests \(<1.09\rangle\) for sodium salt.

**Purity** (1)  Clarity and color of solution—Dissolve 1.0 g of Loxoprofen Sodium Hydrate in 10 mL of water: the solution is clear and colorless or pale yellow. The color is not darker than that of diluted Matching Fluid for Color A (1 in 2).

(2)  Heavy metals \(<1.07\rangle—\)Proceed with 2.0 g of Loxoprofen Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3)  Related substances—Dissolve 1.0 g of Loxoprofen Sodium Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03\rangle\).

Spot 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.07\rangle\) according to the following conditions, and calculate the ratios, \(Q_2\) and \(Q_3\), of the peak area of loxoprofen to that of the internal standard.

\[
M_5 = \frac{M_S \times Q_2}{Q_3} \times 1.089
\]

**Purification**—Amount (mg) of Loxoprofen RS

Internal standard solution—A solution of ethyl benzoate in diluted methanol (3 in 5) (7 in 50,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 222 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecyldimethylsilylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol, water, acetic acid (100) and triethylamine (600:400:1:1).

Flow rate: Adjust the flow rate so that the retention time of loxoprofen is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, loxoprofen and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of loxoprofen to that of the internal standard is not more than 1.0%.

Containers and storage  Containers—Tight containers.
**L-Lysine Acetate**

L-リシン酢酸塩

\[ \text{C}_6\text{H}_{14}\text{N}_2\text{O}_2, \text{C}_2\text{H}_4\text{O}_2: 206.24 \]

(2S)-2,6-Diaminohexanoic acid monoacetate

[L-lysine acetate according to Method 4, and perform the test. Prepare the control solution with 0.35 mL of L-Lysine Acetate, dissolve in 10 mL of water: the solution is colorless and clear.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of L-Lysine Acetate in 10 mL of water: the pH of the solution is between 6.5 and 7.5.

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**Description**

L-Lysine Acetate occurs as white crystals or crystalline powder. It has a characteristic odor and a slightly acid taste.

It is very soluble in water, freely soluble in formic acid, and practically insoluble in ethanol (99.5).

It is deliquescent.

**Identification (1)** Determine the infrared absorption spectrum of L-Lysine Acetate as directed in the potassium bromide disk method under Infrared Spectrophotometry \(2.25\), and compare with the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of L-Lysine Acetate (1 in 20) responds to the Qualitative Tests \(6.09\) (2) for acetate.

**Optical rotation** \(<2.49^\circ\) \([\alpha]_D^20 + 8.5 - +10.0^\circ\) (after drying, 2.5 g, water, 25 mL, 100 mm).

**pH** \(<2.54\) Dissolve 1.0 g of L-Lysine Acetate in 10 mL of water: the pH of the solution is between 6.5 and 7.5.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of L-Lysine Acetate in 10 mL of water: the solution is colorless and clear.

(2) Chloride \(<1.03\) — Perform the test with 0.5 g of L-Lysine Acetate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate \(<1.47\) — Perform the test with 0.6 g of L-Lysine Acetate. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium \(<1.02\) — Perform the test with 0.25 g of L-Lysine Acetate. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals \(<1.07\) — Proceed with 1.0 g of L-Lysine Acetate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron \(<1.10\) — Prepare the test solution with 1.0 g of L-Lysine Acetate according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Weigh accurately about 0.5 g of L-Lysine Acetate, dissolve in 0.5 mL of hydrochloric acid and water to make exactly 100 mL. Pipet 10 mL of this solution, add 0.02 mol/L hydrochloric acid to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately 2.5 mol amounts of L-aspartic acid, L-cystine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-lysine hydrochloride, ammonium chloride, L-histidine and L-arginine, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 1000 mL, and use this solution as the standard stock solution. Pipet 5 mL of this solution, add 0.02 mol/L hydrochloric acid to make exactly 100 mL. Pipet 4 mL of this solution, add 0.02 mol/L hydrochloric acid to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography \(2.01\) according to the following conditions. Based on the peak heights of the amino acids obtained from the sample solution and standard solution, determine the mass of the amino acids other than lysine contained in 1 mL of the sample solution, and calculate the mass percent: the amount of each amino acids other than lysine is not more than 0.1%.

**Operating conditions—**

Detector: A visible spectrophotometer (wavelength: 570 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (Na type) composed with a sulfonated polystyrene copolymer (3 µm particle diameter).

Column temperature: A constant temperature of about 57°C.

Chemical reaction bath temperature: A constant temperature of about 130°C.

Color developing time: About 1 minute.

Mobile phase: Prepare mobile phases A, B, C, D and E according to the following table, and to each phase add 0.1 mL of capric acid.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Mobile amount</th>
<th>Mobile amount</th>
<th>Mobile amount</th>
<th>Mobile amount</th>
<th>Mobile amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>19.80 g</td>
<td>22.00 g</td>
<td>12.80 g</td>
<td>6.10 g</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>6.19 g</td>
<td>7.74 g</td>
<td>13.31 g</td>
<td>26.67 g</td>
<td>—</td>
</tr>
<tr>
<td>C</td>
<td>5.66 g</td>
<td>7.07 g</td>
<td>3.74 g</td>
<td>54.35 g</td>
<td>—</td>
</tr>
<tr>
<td>D</td>
<td>130.00 mL</td>
<td>20 mL</td>
<td>4 mL</td>
<td>100 mL</td>
<td>—</td>
</tr>
<tr>
<td>E</td>
<td>5 mL</td>
<td>5 mL</td>
<td>5 mL</td>
<td>5 mL</td>
<td>—</td>
</tr>
</tbody>
</table>

**Changing mobile phases:** Proceed with 20 µL of the standard solution under the above operating conditions: aspartic acid, threonine, serine, glutamic acid, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, ammonia, histidine and arginine are eluted in this order. Switch over the mobile phases A, B, C, D and E in sequence so that the resolution between the peaks of isoleucine and leucine is not less than 1.2.

Reaction reagents: Dissolve 204 g of lithium acetate dihydrate in water, and add 123 mL of acetic acid (100), 401 mL
of 1-methoxy-2-propanol, and water to make 1000 mL, gas with water for 10 minutes, and use this solution as the solution (I). Separately, to 979 mL of 1-methoxy-2-propanol, add 39 g of ninhydrin, gas with nitrogen for 5 minutes, add 81 mg of sodium borohydride, gas the solution with nitrogen for 30 minutes, and use this solution as solution (II). To 1 volume of the solution (I) add 1 volume of the solution (II). Prepare before use.

Mobile phase flow rate: 0.20 mL per minute.

Reaction reagent flow rate: 0.24 mL per minute.

**System suitability**—

System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, the resolution between the peaks of glycine and alanine is not less than 1.2.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak height of each amino acid in the standard solution is not more than 5.0%, and the relative standard deviation of the retention time is not more than 1.0%.

**Loss on drying** &lt;2.4% Not more than 0.3% (1 g, 80°C, 3 hours).

**Residue on ignition** &lt;2.4% Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.1 g of L-Lysine Acetate, determination in the same manner, and make any necessary correction.

Prepare before use.

Each mL of 0.1 mol/L perchloric acid VS = 10.31 mg of C₆H₁₄N₂O₂.HCl

**Containers and storage** Containers—Tight containers.

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**L-Lysine Hydrochloride**

**L-リシン塩酸塩**

![Chemical Structure](image)

C₆H₁₄N₂O₂.HCl: 182.65

(2S)-2,6-Diaminohexanoic acid monohydrochloride [657-27-2]

L-Lysine Hydrochloride, when dried, contains not less than 98.5% of C₆H₁₄N₂O₂.HCl.

**Description** L-Lysine Hydrochloride occurs as a white powder. It is odorless, and has a slight, characteristic taste. It is freely soluble in water and in formic acid, and practically insoluble in ethanol (95).

**Identification** (1) Determine the infrared absorption spectrum of L-Lysine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry &lt;2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve L-Lysine Hydrochloride in water, evaporate the water to dryness at 60°C, and repeat the test with the residue.

(2) A solution of L-Lysine Hydrochloride (1 in 10) responds to the Qualitative Tests &lt;1.00> for chloride.

**Optical rotation** &lt;2.49> [α]D: +19.0° - +21.5° (after drying, 2 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

**pH** &lt;2.54> Dissolve 1.0 g of L-Lysine Hydrochloride in 10 mL of water: the pH of this solution is between 5.0 and 6.0.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of L-Lysine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate &lt;1.14>—Perform the test with 0.6 g of L-Lysine Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(3) Ammonium &lt;1.02>—Perform the test with 0.25 g of L-Lysine Hydrochloride. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(4) Heavy metals &lt;1.07>—Proceed with 2.0 g of L-Lysine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic &lt;1.11>—Prepare the test solution with 1.0 g of L-Lysine Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 0.10 g of L-Lysine Hydrochloride in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL, pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography &lt;2.03>. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol and ammonia water (28:67:33) to a distance of about 10 cm, and dry the plate at 100°C for 30 minutes. Spray evenly the plate with a solution of ninhydrin in acetone (1 in 50) and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** &lt;2.4% Not more than 1.0% (1 g, 105°C, 3 hours).

**Residue on ignition** &lt;2.4% Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.1 g of L-Lysine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid VS, and heat on a water bath for 30 minutes. After cooling, add 45 mL of acetic acid (100), and titrate &lt;2.50> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 9.132 mg of C₆H₁₄N₂O₂.HCl

**Containers and storage** Containers—Tight containers.
Lysozyme Hydrochloride

リゾチーム塩酸塩

**Structure**

$\text{C}_{616}\text{H}_{963}\text{N}_{193}\text{O}_{182}\text{S}_{10} \cdot \text{xHCl}$

[12650-88-3, egg white lysozyme]

Lysozyme Hydrochloride is a hydrochloride of a basic polypeptide obtained from albumen of hen's egg, and has an activity to hydrolyze mucopolysaccharides.

It contains not less than 0.9 mg (potency) of lysozyme per mg, calculated on the dried basis.

**Description** Lysozyme Hydrochloride occurs as white, crystals, or crystalline or amorphous powder. It is freely soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic. The pH of a solution of Lysozyme Hydrochloride (3 in 200) is between 3.0 and 5.0.

**Identification** (1) To 5 mL of a solution of Lysozyme Hydrochloride in acetate buffer solution, pH 5.4 (1 in 500) add 1 mL of ninhydrin TS, and heat for 10 minutes: a blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Lysozyme Hydrochloride in acetate buffer solution, pH 5.4 (1 in 500) add 1 mL of a standard solution (1) and the standard solution (2) in the same manner as the sample solution.

- Amount [mg (potency)] of lysozyme per mg, calculated on the dried basis
  \[ M_S = \frac{2M_T}{M_T} \times \frac{(A_{S1} - A_T)}{(A_{S2} - A_T) + 1} \]

- $M_S$: Amount (mg) of Lysozyme RS, calculated on the dried basis.
- $M_T$: Amount (mg) of the sample, calculated on the dried basis.

**Containers and storage** Containers—Tight containers.

**Macrogol 400**

Polyethylene Glycol 400

マクロゴール 400

Macrogol 400 is a polymer of ethylene oxide and water, represented by the formula $\text{HOCH}_2\text{CH}_2\text{O}_n\text{CH}_2\text{OH}$, in which the value of $n$ ranges from 7 to 9.

**Description** Macrogol 400 occurs as a clear, colorless and viscous liquid. It has no odor or a slight, characteristic odor. It is miscible with water, with methanol, with ethanol (95) and with pyridine.

It is soluble in diethyl ether.

It is slightly hygroscopic.

Congealing point: 4–8°C

Specific gravity $d_{20}^0$: 1.110 – 1.140

**Identification** Dissolve 50 mg of Macrogol 400 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid $n$-hydrate (1 in 10): a yellow-green precipitate is formed.

**Purity** (1) Acidity—Dissolve 5.0 g of Macrogol 400 in 20 mL of neutralized ethanol, and add 2 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: the solution is red in color.

(2) Ethylene glycol and diethylene glycol—Dissolve 4.0 g of Macrogol 400 in water to make exactly 10 mL, and use this solution as the sample solution. Weigh accurately about 50 mg each of ethylene glycol and diethylene glycol, dissolve 2 mL of this solution, add phosphate buffer solution, pH 6.2 to them to make exactly 50 mL, and use these solutions as the standard solution (1) and the solution (2), respectively. Keep the sample solution and the standard solutions in an ice-bath. Pipet 4 mL of substrate solution for lysozyme hydrochloride, previously warmed in a water bath of 35°C for about 5 minutes, add exactly 100 µL of the sample solution, previously warmed in a water bath of 35°C for about 3 minutes, and allow to stand at 35°C for exactly 10 minutes, then add exactly 0.5 mL of 1 mol/L hydrochloric acid TS, and immediately shake. Determine the absorbance under Ultraviolet-visible Spectrophotometry $<2.24>$, $A_T$, of this solution at 640 nm, using water as the blank. Determine the absorbances, $A_{S1}$ and $A_{S2}$, of the solutions obtained with the standard solution (1) and the standard solution (2) in the same manner as the sample solution.

Amount [mg (potency)] of lysozyme per mg, calculated on the dried basis

- $M_S = \frac{2M_T}{M_T} \times \frac{(A_{S1} - A_T)}{(A_{S2} - A_T) + 1}$
- $M_T$: Amount (mg) of the sample, calculated on the dried basis.

**Containers and storage** Containers—Tight containers.
in water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Gas Chromatography in the following conditions: Determine the peak heights, $H_{Sa}$ and $H_{Sb}$, of ethylene glycol of each solution, and the peak heights, $H_{Ta}$ and $H_{Tb}$, of diethylene glycol, and calculate the amount of ethylene glycol and diethylene glycol: the sum of the contents of ethylene glycol and diethylene glycol is not more than 0.25%.

$$M_{Sa} = M_s \times \frac{H_{Ta}}{H_{Sa}} \times \frac{1}{10}$$

$$M_{Sb} = M_s \times \frac{H_{Tb}}{H_{Sb}} \times \frac{1}{10}$$

$M_s$: Amount (mg) of ethylene glycol for gas chromatography

$M_{Sa}$: Amount (mg) of ethylene glycol for gas chromatography

Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A column about 3 mm in inside diameter and about 1.5 m in length, packed with siliceous earth for gas chromatography, 150 to 180 μm in particle diameter, coated with β-sorbil at the ratio of 12%.
Column temperature: A constant temperature of about 165°C.
Carrier gas: Nitrogen or helium.
Flow rate: Adjust the flow rate so that the retention time of diethylene glycol is about 8 minutes.
Selection of column: Proceed with 2 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column clearly dividing peaks of ethylene glycol and diethylene glycol in this order.
Detection sensitivity: Adjust the detection sensitivity so that the peak height of diethylene glycol obtained from 2 μL of the standard solution composes about 80% of the full scale.

Average molecular mass

Add 42 g of phthalic anhydride to 300 mL of freshly distilled pyridine, exactly measured, in a 1-L light-resistant glass-stoppered bottle. Shake the bottle vigorously to dissolved the solid, and allow to stand for 16 hours or more. Pipet 25 mL of this solution into an about 200-mL glass-stoppered pressure bottle. Add about 1.5 g of Macrogol 400, accurately weighed, stopper the bottle, wrap it securely with strong cloth, and immerse in a water bath, having a temperature of 98 ± 2°C, to the level so that the mixture in the bottle soaks completely in water. Maintain the temperature of the bath at 98 ± 2°C for 30 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination.

$$M = \frac{(M \times 4000) / (a - b)}{V}$$

$M$: Amount (g) of sample
$a$: Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the blank determination
$b$: Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the test of the sample

Average molecular mass is between 380 and 420.

Water $<2.48$ Not more than 1.0% (2 g, volumetric titration, direct titration).

Residue on ignition $<2.48$ Not more than 0.1% (1 g).

Containers and storage—Containers—Tight containers.

Macrogol 1500

マクロゴール 1500

Macrogol 1500 is a mixture containing equal amounts of lower and higher polymers of ethylene oxide and water, represented by the formula $\text{HOCH}_2\text{(CH}_2\text{OCH}_2)n\text{CH}_2\text{OH}$, in which the value of $n$ is 5 or 6 for the lower polymers and from 28 to 36 for the higher.

Description

Macrogol 1500 occurs as a white, smooth petrolatum-like solid. It is odorless or has a faint, characteristic odor.

It is very soluble in water, in pyridine and in diphenyl ether, freely soluble in methanol, sparingly soluble in ethanol (95), very slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

Congealing point: 37 - 41°C

Identification

Dissolve 50 mg of Macrogol 1500 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid n-hydrate (1 in 10): a yellow-green precipitate is formed.

pH $<2.50$ Dissolve 1.0 g of Macrogol 1500 in 20 mL of water: the pH of the solution is between 4.0 and 7.0.

Purity

Clarity and color of solution—Dissolve 5.0 g of Macrogol 1500 in 50 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 5.0 g of Macrogol 1500 in 20 mL of neutralized ethanol, and add 2 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: the solution is red in color.

(3) Ethylene glycol and diethylene glycol—Place 50.0 g of Macrogol 1500 in a distilling flask, add 75 mL of dimethyl ether, warm to dissolve if necessary, distill slowly under a reduced pressure of 0.13 to 0.27 kPa and take 25 mL of the distillate in a 100-mL container with 1-mL graduation. To the distillate add exactly 20 mL of water, shake vigorously, cool in ice water, congeal the diethyl ether, and filtrate into a 25-mL volumetric flask. Wash the residue with 5.0 mL of ice-cold water, combine the washings with the filtrate, warm to room temperature, and add water to make 25 mL. Transfer this solution to a glass-stoppered flask, shake with 25.0 mL of freshly distilled acetonitrile, and use this solution as the sample solution. Separately, to 62.5 mg of diethylene glycol add a mixture of water and freshly distilled acetonitrile (1:1) to make exactly 25 mL, and use this solution as the standard solution. Take exactly 10 mL each of the sam-
Macrogol 4000

Polyethylene Glycol 4000

Macrogol 4000 is a polymer of ethylene oxide and water, represented by the formula HOCH₂(CH₂OCH₂)ₙCH₂OH, in which the value of n ranges from 59 to 84.

Description
Macrogol 4000 is a white, paraffin-like solid, occurring as flakes or powder. It is odorless or has a faint, characteristic odor.

It is very soluble in water, freely soluble in methanol and in pyridine, and practically insoluble in ethanol (99.5) and in diethyl ether.

Congealing point: 53 – 57°C

Identification
Dissolve 50 mg of Macrogol 4000 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid n-hydrate (1 in 10): a yellow-green precipitate is formed.

pH < 2.54
Dissolve 1.0 g of Macrogol 4000 in 20 mL of water: the pH of this solution is between 4.0 and 7.5.

Purity (1)
Clarity and color of solution—A solution of 5.0 g of Macrogol 4000 in 50 mL of water is clear and colorless.

(2) Acidity—Dissolve 5.0 g of Macrogol 4000 in 20 mL of neutralized ethanol by warming, cool, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 1 drop of phenolphthalein TS: the color of the solution is red.

Average molecular mass
Weigh accurately about 12.5 g of Macrogol 4000, transfer to an about 200-mL glass-stoppered pressure bottle, add about 25 mL of pyridine, dissolve by warming, and allow to cool. Separately, pipet 300 mL of freshly distilled pyridine into a 1000-mL light-resistant, glass-stoppered bottle, add 42 g of phthalic anhydride, dissolve with vigorous shaking, and allow to stand for 16 hours or more. Pipet 25 mL of this solution, transfer to the former pressure bottle, stopper the bottle tightly, wrap it securely with strong cloth, and immerse in a water bath, previously heated at 98 ± 2°C, to the level so that the mixture in the bottle soaks completely in water. Maintain the temperature of the bath at 98 ± 2°C for 30 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate < 2.50 with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination.

Average molecular mass = \( \frac{M \times 4000}{(a - b)} \)

M: Amount (g) of sample
a: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the blank determination
b: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the test of the sample

Average molecular mass is between 2600 and 3800.

Water < 2.48
Not more than 1.0% (2 g, volumetric titration, direct titration).

Residue on ignition < 2.44
Not more than 0.1% (1 g).

Containers and storage
Containers—Well-closed containers.

Macrogol 6000

Polyethylene Glycol 6000

Macrogol 6000 is a polymer of ethylene oxide and water, represented by the formula HOCH₂(CH₂OCH₂)ₙCH₂OH, in which the value of n ranges from 165 to 210.

Description
Macrogol 6000 is a white, paraffin-like solid, occurring as flakes or powder. It is odorless or has a faint, characteristic odor.

It is very soluble in water, freely soluble in pyridine, and practically insoluble in methanol, in ethanol (95), in ethanol (99.5) and in diethyl ether.

Congealing point: 56 – 61°C

Identification
Dissolve 50 mg of Macrogol 6000 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid n-hydrate (1 in 10): a yellow-green precipitate is formed.

pH < 2.54
Dissolve 1.0 g of Macrogol 6000 in 20 mL of water: the pH of this solution is between 4.5 and 7.5.

Purity (1)
Clarity and color of solution—A solution of 5.0 g of Macrogol 6000 in 50 mL of water is clear and colorless.

(2) Acidity—Dissolve 5.0 g of Macrogol 6000 in 20 mL of neutralized ethanol by warming, cool, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 1 drop of phenolphthalein TS: the color of the solution is red.

Average molecular mass
Weigh accurately about 12.5 g of Macrogol 6000, transfer to an about 200-mL glass-stoppered pressure bottle, add about 25 mL of pyridine, dissolve by warming, and allow to cool. Separately, pipet 300 mL of freshly distilled pyridine into a 1000-mL light-resistant, glass-stoppered bottle, add 42 g of phthalic anhydride, diss-
solve with vigorous shaking, and allow to stand for 16 hours or more. Pipet 25 mL of this solution, transfer to the former pressure bottle, stopper the bottle tightly, wrap it securely with strong cloth, and immerse in a water bath, previously heated at 98 ± 2°C, to the level so that the mixture in the bottle soaks completely in water. Maintain the temperature of the bath at 98 ± 2°C for 30 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate \( <2.50^\circ \) with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination in the same manner.

Average molecular mass = \( (M \times 4000)/(a - b) \)

\( M \): Amount (g) of sample
\( a \): Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the blank determination
\( b \): Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the test of the sample

Average molecular mass is between 7300 and 9300.

Water \( <2.48^\circ \) Not more than 1.0% (2 g, volumetric titration, direct titration).

Residue on ignition \( <2.44^\circ \) Not more than 0.2% (1 g).

Containers and storage Containers—Well-closed containers.

Macrogol 20000

Polyethylene Glycol 20000

マクロゴール 20000

Macrogol 20000 is a polymer of ethylene oxide and water, represented by the formula \( \text{HOCH}_2\text{(CH}_2\text{OCH}_2)_n\text{CH}_2\text{OH} \), in which the value of \( n \) lies between 340 and 570.

Description Macrogol 20000 occurs as white, paraffin-like flakes or powder. It is odorless or has a faint, characteristic odor.

It is freely soluble in water and in pyridine, and practically insoluble in methanol, in ethanol (95), in dehydrated diethyl ether, in petroleum benzine and in macrogol 400.

Congealing point: 56 – 64°C

Identification Dissolve 50 mg of Macrogol 20000 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid \( n \)-hydrate (1 in 10): a yellow-green precipitate is formed.

pH \( <2.54^\circ \) Dissolve 1.0 g of Macrogol 20000 in 20 mL of water: the pH of this solution is between 4.5 and 7.5.

Purity (1) Clarity and color of solution—Dissolve 5.0 g of Macrogol 20000 in 50 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 5.0 g of Macrogol 20000 in 20 mL of neutralized ethanol by warming, cool, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 1 drop of phenolphthalein TS: the color of the solution is red.

Average molecular mass Weigh accurately about 15 g of Macrogol 20000, transfer to an about 200-mL glass-stoppered pressure bottle, add about 25 mL of pyridine, dissolve by warming, and allow to cool. Separately, pipet 300 mL of freshly distilled pyridine into a 1000-mL light-resistant glass-stoppered bottle, add 42 g of phthalic anhydride, dissolve with vigorous shaking, and allow to stand for 16 hours or more. Pipet 25 mL of this solution, transfer to the former pressure bottle, stopper the bottle tightly, wrap it securely with strong cloth, and immerse in a water bath, having a temperature of 98 ± 2°C, to the same depth as the mixture in the bottle. Maintain the temperature of the bath at 98 ± 2°C for 60 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate \( <2.50^\circ \) with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination.

Average molecular mass = \( (M \times 4000)/(a - b) \)

\( M \): Amount (g) of sample
\( a \): Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the blank determination
\( b \): Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the test of the sample

Average molecular mass is between 15000 and 25000.

Water \( <2.48^\circ \) Not more than 1.0% (2 g, volumetric titration, direct titration).

Residue on ignition \( <2.44^\circ \) Not more than 0.2% (1 g).

Containers and storage Containers—Well-closed containers.

Macrogol Ointment

Polyethylene Glycol Ointment

マクロゴール軟膏

To make 1000 g Melt Macrogol 4000 and Macrogol 400 by warming on a water bath at 65°C, and mix well until it congeals. Less than 100 g of Macrogol 4000 or Macrogol 400 may be replaced by an equal amount of Macrogol 400 or Macrogol 4000 to prepare 1000 g of a proper soft ointment.

Method of preparation

| Macrogol 4000 | 500 g |
| Macrogol 400  | 500 g |

Description Macrogol Ointment is white in color. It has a faint, characteristic odor.

Identification Dissolve 50 mg of Macrogol Ointment in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, filter if necessary, and add 1 mL of a solution of phosphomolybdic acid \( n \)-hydrate (1 in 10) to the filtrate: a yellow-green precipitate is formed.
Magnesium Carbonate

Magnesium Carbonate is a basic hydrated magnesium carbonate or a normal hydrated magnesium carbonate.

Magnesium Carbonate contains not less than 40.0% and not more than 44.0% of magnesium oxide (MgO: 40.30).

“Heavy magnesium carbonate” may be used as commonly used name for Magnesium Carbonate which shows the height of the precipitate below the 12.0-mL graduation line in the Precipitation test.

**Description**
Magnesium Carbonate occurs as white, friable masses or powder. It is odorless.

It is practically insoluble in water, in ethanol (95), in diethyl ether and in 1-propanol.

It dissolves in dilute hydrochloric acid with effervescence.

Its saturated solution is alkaline.

**Purity**

(1) Soluble salts—To 2.0 g of Magnesium Carbonate add 40 mL of 1-propanol and 40 mL of water, heat to boil with constant stirring, cool, neutralize with sodium hydroxide TS, and filter, if necessary: the solution responds to the Qualitative Tests \(<1.09\) for magnesium salt.

(2) Magnesium Carbonate responds to the Qualitative Tests \(<1.09\) (1) for carbonate.

**Assay**

Weigh accurately about 0.4 g of Magnesium Carbonate, dissolve in 10 mL of water and 3.5 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, 2 mL of dilute acetic acid, 1 drop of ammonia TS, filter, if necessary, wash the filter paper with water, combine the washings with the filtrate, and add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 10 mL of dilute hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 3.0 mL of Standard Lead Solution, and dilute with water to make 50 mL (not more than 30 ppm).

(3) Iron \(<1.10\)—Prepare the test solution with 0.10 g of Magnesium Carbonate according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 200 ppm).

(4) Arsenic \(<1.11\)—Prepare the test solution with 0.40 g of Magnesium Carbonate, previously moistened with 1.5 mL of water, add 3.5 mL of dilute hydrochloric acid, and perform the test (not more than 5 ppm).

(5) Calcium oxide—Weigh accurately about 0.6 g of Magnesium Carbonate, and dissolve in 35 mL of water and 6 mL of dilute hydrochloric acid. Add 250 mL of water and 5 mL of a solution of L-tartaric acid (1 in 5), then add 10 mL of a solution of 2,2',2"-nitrilotrisethanol (3 in 10) and 10 mL of 8 mol/L potassium hydroxide TS, allow to stand for 5 minutes, and titrate \(<2.50\) with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes form red-purple to blue (indicator: 0.1 g of NN indicator). Perform a blank determination, and make any necessary correction.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS

\[= 0.5608 \text{ mg of CaO} \]

The content of calcium oxide (CaO: 56.08) is not more than 0.6%.

(6) Acid-insoluble substances—Mix 5.0 g of Magnesium Carbonate and 75 mL of water, add 10 mL of hydrochloric acid dropwise while stirring, boil for 5 minutes, and cool. Collect the insoluble residue using filter paper for quantitative analysis, wash well with water until the last washing shows no turbidity with silver nitrate TS, and ignite the residue together with the filter paper: the mass of the residue is not more than 2.5 mg.

**Precipitation test**
Transfer 1.0 g of Magnesium Carbonate, previously sifted through a No. 100 (150 μm) sieve to a glass-stoppered measuring cylinder with a 50-mL graduation line at 150 mm from the bottom, and add water to make 50 mL. Shake vigorously for exactly 1 minute, allow to stand for 15 minutes, and measure the height of the precipitate (in graduation ml).

**Containers and storage**
Containers—Tight containers.
Magnesium Oxide

酸化マグネシウム

MgO: 40.30

Magnesium Oxide, when ignited, contains not less than 96.0% of MgO.

When 5 g of Magnesium Oxide has a volume not more than 30 mL, it may be labeled heavy magnesium oxide.

Description Magnesium Oxide occurs as a white powder or granules. It is odorless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid.

It absorbs moisture and carbon dioxide in air.

Identification A solution of Magnesium Oxide in dilute hydrochloric acid (1 in 50) responds to the Qualitative Tests for magnesium salt.

Purity (1) Alkali and soluble salts—Transfer 2.0 g of Magnesium Oxide to a beaker, add 100 mL of water, cover the beaker with a watch-glass, heat on a water bath for 5 minutes, and filter immediately. After cooling, to 50 mL of the filtrate add 2 drops of methyl red TS and 2.0 mL of 0.05 mol/L sulfuric acid VS: a red color develops. Evaporate 25 mL of the remaining filtrate to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 10 mg.

(2) Carbonate—Boil 0.10 g of Magnesium Oxide with 5 mL of water, cool, and add 5 mL of acetic acid (31): almost no effervescence occurs.

(3) Heavy metals <1.07>—Dissolve 1.0 g of Magnesium Oxide in 20 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, add 1 drop of phenolphthalein TS, neutralize with ammonia TS, add 2 mL of dilute acetic acid, and filter, if necessary. Wash the filter paper with water, add water to the combined washing and the filtrate to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 20 mL of dilute hydrochloric acid add 1 drop of phenolphthalein TS, neutralize with ammonia TS, and add 2 mL of dilute acetic acid, 4.0 mL of Standard Lead Solution and water to make 50 mL (not more than 40 ppm).

(4) Iron <1.10>—Prepare the test solution with 40 mg of Magnesium Oxide according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 500 ppm).

(5) Calcium oxide—Weigh accurately about 0.25 g of Magnesium Oxide, previously ignited, dissolve in 6 mL of dilute hydrochloric acid by heating. Cool, add 300 mL of water and 3 mL of a solution of L-tartaric acid (1 in 5), then add 10 mL of a solution of 2,2',2'-nitrolotrisethanol (3 in 10) and 10 mL of 8 mol/L potassium hydroxide VS, allow to stand for 5 minutes, and titrate 2.50 with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue (indicator: 0.1 g of NN indicator). Perform a blank determina-

The figures are in mm.

A: Distilling flask of about 300-mL capacity.
B: Steam generator of about 1000-mL capacity, containing a few boiling lips to prevent bumping
C: Condenser
D: Receiver: 200-mL volumetric flask
E: Steam-introducing tube having an internal diameter of about 8 mm
F: G: Rubber tube with a clamp
H: Thermometer

The mass of calcium oxide (CaO: 56.08) is not more than 1.5%.

(6) Arsenic <1.11>—Dissolve 0.20 g of Magnesium Oxide in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 10 ppm).

(7) Acid-insoluble substances—Mix 2.0 g of Magnesium Oxide with 75 mL of water, add 12 mL of hydrochloric acid dropwise, while shaking, and boil for 5 minutes. Collect the insoluble residue using filter paper for quantitative analysis, wash well with water until the last washing shows no turbidity with silver nitrate TS, and ignite the residue together with the filter paper: the mass of the ignited residue does not more than 2.0 mg.

(8) Fluoride—(i) Apparatus: Use a hard glass apparatus as illustrated in the figure. Ground-glass joints may be used.

(i) Procedure: Transfer 5.0 g of Natural Aluminum Silicate to the distilling flask A with the aid of 20 mL of water, add about 1 g of glass wool and 50 mL of diluted purified sulfuric acid (1 in 2), and connect A to the distillation apparatus, previously washed with steam streamed through the steam introducing tube E. Connect the condenser C with the receiver D containing 10 mL of 0.01 mol/L sodium hydroxide VS and 10 mL of water so that the lower end of C is immersed in the solution. Heat A gradually until the temperature of the solution in A reaches 130°C, then open the rub-
ber tube F, close the rubber tube G, boil water in the steam
generator B vigorously, and introduce the generated steam
into F. Simultaneously, heat A, and maintain the tempera-
ture of the solution in A between 135°C and 145°C. Adjust
the distilling rate to about 10 mL per minute. Collect about
170 mL of the distillate, then stop the distillation, wash C
with a small quantity of water, combine the washings with
the distillate, add water to make exactly 200 mL, and use this
solution as the test solution. Perform the test with the test
solution as directed in the procedure of determination for
fluoride under Oxygen Flask Combustion Method <1.06>.
No corrective solution is used in this procedure. The content
of fluoride (F) is not more than 0.08%.

Amount (mg) of fluoride (F: 19.00) in the test solution
= amount (mg) of fluoride in 5 mL of
the standard solution
× A1/As × 200/V

Loss on ignition <2.43> Not more than 10% (0.25 g, 900°C, constant mass).

Assay Ignite Magnesium Oxide to constant mass at 900°C, weigh accurately about 0.2 g of the residue, dissolve in 10
mL of water and 4.0 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Pipet 25 mL of this solution,
add 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <2.50> with
0.05 mol/L disodium dihydrogen ethylenediamine tetra-
acetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination, and
make any necessary correction.

From the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetracetate VS consumed, deduct the
volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetracetate VS corresponding to the content of calcium
oxide (CaO) obtained in the Purity (5).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetracetate VS
= 2.015 mg of MgO
Each mg of calcium oxide (CaO)
= 0.36 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetracetate VS

Containers and storage Containers—Tight containers.

Magnesium Silicate

ケイ酸マグネシウム

Magnesium Silicate contains not less than 45.0% of silicon dioxide (SiO2: 60.08) and not less than 20.0% of magnesium oxide (MgO: 40.30), and the ratio of percentage (%) of magnesium oxide to silicon dioxide is not less than 2.2 and not more than 2.5.

Description Magnesium Silicate occurs as a white, fine powder. It is odorless and tasteless.
It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Identification (1) Mix 0.5 g of Magnesium Silicate with
10 mL of dilute hydrochloric acid, filter, and neutralize the
filtrate with ammonia TS: the solution responds to the
Qualitative Tests <1.09> for magnesium salt.

(2) Prepare a bead by fusing ammonium sodium hydro-
genphosphate tetrahydrate on a platinum loop. Place the
bead in contact with Magnesium Silicate, and fuse again: an
infusible matter appears in the bead, which changes to an
opaque bead with a web-like structure upon cooling.

Purity (1) Soluble salts—Add 150 mL of water to 10.0 g
of Magnesium Silicate, heat on a water bath for 60 minutes
with occasional shaking, then cool, dilute with water to 150
mL, and centrifuge. Dilute 75 mL of the resultant transpar-
ent liquid with water to 100 mL, and use this solution as the
sample solution. Evaporate 25 mL of the sample solution
on a water bath to dryness, and ignite the residue at 700°C for
2 hours: the mass of the ignited residue is not more than
0.02 g.

(2) Alkalinity—To 20 mL of the sample solution ob-
tained in (1) add 2 drops of phenolphthalein TS and 1.0 mL
of 0.1 mol/L hydrochloric acid VS: no color develops.

(3) Chloride <1.07>—Take 10 mL of the sample solution
obtained in (1), add 6 mL of dilute nitric acid, dilute with
water to 50 mL, and perform the test using this solution as
the test solution. Prepare the control solution with 0.75 mL
of 0.01 mol/L hydrochloric acid VS (not more than 0.053%).

(4) Sulfate <1.14>—To the residue obtained in (1) add
about 3 mL of dilute hydrochloric acid, and heat on a water
bath for 10 minutes. Add 30 mL of water, filter, wash the
residue on the filter with water, combine the washings with
the filtrate, and dilute to 50 mL with water. To 4 mL of
the solution add 1 mL of dilute hydrochloric acid and water
to make 50 mL. Perform the test using this solution as the test
solution. Prepare the control solution with 1.0 mL of 0.005
mol/L sulfuric acid VS (not more than 0.480%).

(5) Heavy metals <1.07>—To 1.0 g of Magnesium Silicate
add 20 mL of water and 3 mL of hydrochloric acid, and boil
for 2 minutes. Filter, and wash the residue on the filter with
two 5-mL portions of water. Evaporate the combined filtrate
and washings on a water bath to dryness, add 2 mL of dilute
acetic acid to the residue, warm until solution is complete,
filter, if necessary, add water to make 50 mL, and perform
the test using this solution as the test solution. Prepare the
control solution with 3.0 mL of Standard Lead Solution, 2
mL of dilute acetic acid and water to make 50 mL (not more
than 30 ppm).

(6) Arsenic <1.11>—To 0.4 g of Magnesium Silicate add
5 mL of dilute hydrochloric acid, heat gently to boiling while
shaking well, cool rapidly, and centrifuge. Mix the residue
with 5 mL of dilute hydrochloric acid with shaking, centri-
fuge, then add 10 mL of water to the residue, and repeat
the extraction in the same manner. Concentrate the combined
extracts on a water bath to 5 mL. Use this solution as the test
solution, and perform the test (not more than 5 ppm).

Loss on ignition <2.43> Not more than 34% (0.5 g, 850°C, 3 hours).

Acid-consuming capacity <6.04> Place about 0.2 g of Mag-
nesium Silicate, accurately weighed, in a glass-stoppered
flask, add exactly 30 mL of 0.1 mol/L hydrochloric acid VS
and 20 mL of water, shake at 37 ± 2°C for 1 hour, and
cool. Pipet 25 mL of the supernatant liquid, and titrate
<2.50> the excess hydrochloric acid, while stirring well, with
0.1 mol/L sodium hydroxide VS until the pH becomes 3.5. 1 g of Magnesium Silicate, calculated on the anhydrous basis by making allowance for the observed loss on ignition determined as directed in the preceding Loss on ignition, consumes not less than 140 mL and not more than 160 mL of 0.1 mol/L hydrochloric acid VS.

**Assay** (1) Silicon dioxide—Weigh accurately about 0.7 g of Magnesium Silicate, add 10 mL of 0.5 mol/L sulfuric acid TS, evaporate on a water bath to dryness, add 25 mL of water to the residue, and heat on a water bath for 15 minutes with occasional stirring. Filter the supernatant liquid through filter paper for assay, add 25 mL of hot water to the residue, stir, and decant the supernatant liquid on the filter paper to filter. Wash the residue in the same manner with two 25-mL portions of hot water, transfer the residue onto the filter paper, and wash with hot water until the last washing does not respond to the Qualitative Tests <1.09> (1) for sulfate. Place the residue and the filter paper in a platinum crucible, incinerate with strong heating, and ignite between 775°C and 825°C for 30 minutes, then cool, and weigh the residue as a (g). Moisten the residue with water, and add 6 mL of hydrofluoric acid and 3 drops of sulfuric acid. Evaporate to dryness, ignite for 5 minutes, cool, and weigh the residue as b (g).

\[
\text{Mass of the sample} = \frac{a - b}{M} \times 100
\]

(2) Magnesium oxide—Weigh accurately about 0.3 g of Magnesium Silicate, transfer to a 50-mL conical flask, add 10 mL of 0.5 mol/L sulfuric acid VS, and heat on a water bath for 15 minutes. Cool, transfer to a 100-mL volumetric flask, wash the conical flask with water, add the washings to the volumetric flask, dilute with water to 100 mL, and filter. Pipet 50 mL of the filtrate, shake with 50 mL of water and 5 mL of diluted 2.2′,2″-nitroilrotisethanol (1 in 2), add 2.0 mL of ammonium TS and 10 mL of ammonium-ammonium chloride buffer solution, pH 10.7, and titrate \( <2.50^\circ >\) with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 2.015 mg of MgO

(3) Ratio of percentage (%) of magnesium oxide (MgO) to silicon dioxide (SiO2)—Calculate the quotient from the percentages obtained in (1) and (2).

**Containers and storage** Containers—Well-closed containers.

**Magnesium Stearate**

ステアリン酸マグネシウム

Magnesium Stearate consists chiefly magnesium salts of stearic acid \((C_{17}H_{35}O_2: 284.48)\) and palmitic acid \((C_{15}H_{31}O_2: 256.42)\).

It contains, when dried, not less than 4.0% and not more than 5.0% of magnesium (Mg: 24.31).

**Description** Magnesium Stearate occurs as a white, light, bulky powder.

It is smooth to the touch and sticky to the skin. It has no odor or a faint, characteristic odor.

It is practically insoluble in water and in ethanol (95).

**Identification** (1) Mix 5.0 g of Magnesium Stearate with 50 mL of peroxide-free diethyl ether, 20 mL of dilute nitric acid, and 20 mL of water in a round-bottom flask, and heat to dissolve completely under a reflux condenser. After cooling, transfer the contents of the flask to a separator, shake, allow the layers to separate, and transfer the aqueous layer to a flask. Extract the diethyl ether layer with two 4-mL portions of water, and combine these extracts to the main aqueous extract. After washing the combined aqueous extract with 15 mL of peroxide-free diethyl ether, transfer to a 50-mL volumetric flask, add water to make exactly 50 mL, mix, and use this solution as the sample solution: the sample solution corresponds to the Qualitative Tests <1.09> for magnesium.

(2) The retention times of the peaks corresponding to stearyl stearate and methyl palmitate in the chromatogram of the sample solution correspond to those of methyl stearate and methyl palmitate in the chromatogram of the system suitability solution, as obtained in the Purity (5).

**Purity** (1) Acidity or alkalinity—Heat 1.0 g of Magnesium Stearate in 20 mL of freshly boiled and cooled water on a water bath for 1 minute while shaking, and filter after cooling. To 10 mL of the filtrate add 0.05 mL of bromothymol blue TS, and add exactly 0.05 mL of 0.1 mol/L hydrochloric acid VS or 0.1 mol/L sodium hydroxide VS: the color of the solution changes.

(2) Chloride <1.03>—Perform the test with 10.0 mL of the sample solution obtained in Identification (1). Prepare the control solution with 1.40 mL of 0.02 mol/L hydrochloric acid VS (not more than 0.10%).

(3) Sulfate <1.14>—Perform the test with 10.0 mL of the sample solution obtained in Identification (1). Prepare the control solution with 10.2 mL of 0.01 mol/L sulfuric acid VS (not more than 1.0%).

(4) Heavy metals <1.07>—Heat 1.0 g of Magnesium Stearate weakly first, then incinerate at about 500 ± 25°C. After cooling, add 2 mL of hydrochloric acid, evaporate on a water bath to dryness, add 20 mL of water and 2 mL of dilute acetic acid to the residue, and heat for 2 minutes. After cooling, filter this solution through a filter paper, wash the filter paper with 15 mL of water, and combine the washing with the filtrate. To the filtrate add water to make 50 mL, and perform the test with this solution as the test solution. Prepare the control solution as follows: evaporate 2 mL of hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(5) Relative content of stearic acid and palmitic acid—Transfer exactly 0.10 g of Magnesium Stearate to a small conical flask fitted with a reflux condenser. Add 5.0 mL of boron trifluoride-methanol TS, mix, and reflux for about 10 minutes to dissolve the solids. Add 4.0 mL of heptane through the condenser, and reflux for about 10 minutes. After cooling, add 20 mL of saturated sodium chloride solution, shake, and allow the layers to separate. Transfer the heptane layer through about 0.1 g of anhydrous sodium sulfate, previously washed with heptane, to another
flask. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute with heptane to volume, mix, and use this solution as the sample solution. Perform the test with 1 μL of the sample solution as directed under Gas chromatography according to the following conditions, and determine the area, \( A \), of the methyl stearate peak and the total of the areas, \( B \), of all fatty acid ester peaks. Calculate the percentage of stearic acid in the fatty acid fraction of Magnesium Stearate by the following formula.

\[
\text{Content} (\%) \text{ of stearic acid} = \frac{A}{B} \times 100
\]

Similarly, calculate the percentage of palmitic acid in Magnesium Stearate. The methyl stearate peak, and the total of the methyl stearate and methyl palmitate peaks are not less than 40% and not less than 90% of the total area of all fatty acid ester peaks, respectively, in the chromatogram.

**Operating conditions—**

Detector: A hydrogen flame-ionization detector maintained at a constant temperature of about 260°C.

Sample injection port: A splitless injection system maintained at a constant temperature of about 220°C.

Column: A fused silica capillary column 0.32 mm in inside diameter and 30 m in length, the inside coated with a 0.5-μm layer of polyethylene glycol 15000-diepoxyde for gas chromatography.

Column temperature: Maintain at 70°C for 2 minutes after injection, then program to increase the temperature at the rate of 5°C per minute to 240°C and to maintain this temperature for 5 minutes.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of methyl stearate is about 32 minutes.

Split ratio: Splitless.

Time span of measurement: About 1.5 time as long as the retention time of methyl stearate beginning after the solvent peak.

**System suitability—**

Test for required detection: Place exactly 50 mg each of stearic acid for gas chromatography and palmitic acid for gas chromatography, each previously dried in a desiccator (silica gel) for 4 hours, in a small conical flask fitted with a reflux condenser. Add 5.0 mL of boron trifluoride-methanol TS, mix, and proceed in the same manner as directed for the preparation of the sample solution, and use the solution so obtained as the solution for system suitability test. To exactly 1 mL of the solution add heptane to make exactly 10 mL. Confirm that the peak area of methyl stearate obtained from 1 μL of this solution is equivalent to 5 to 15% of that from 1 μL of the solution for system suitability test.

System performance: When the procedure is run with 1 μL of the solution for system suitability test under the above operating conditions, methyl palmitate and methyl stearate are eluted in this order, with the relative retention time of methyl palmitate to methyl stearate being about 0.86, and with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of methyl palmitate and methyl stearate are not more than 6.0%, respectively, and the relative standard deviation of the ratios of the peak area of methyl palmitate to methyl stearate is not more than 1.0%.

**Loss on drying** Not more than 6.0% (2 g, 105°C, constant mass).

**Microbial limit** The acceptance criteria of TAMC and TYMC are 10³ CFU/g and 5 × 10³ CFU/g, respectively. *Salmonella* and *Escherichia coli* are not observed.

**Assay** Transfer about 0.5 g of previously dried Magnesium Stearate, accurately weighed, to a 250-mL flask, add 50 mL of a mixture of 1-butanol and ethanol (99.5) (1:1), 5 mL of ammonia solution (28), 3 mL of ammonium chloride buffer solution, pH 10, 30.0 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, and 1 to 2 drops of eriochrome black T TS, and mix. Heat at 45°C to 50°C to make the solution clear, and after cooling, titrate mg of Mg to the excess disodium dihydrogen ethylenediamine tetraacetate with 0.1 mol/L zinc sulfate VS until the solution changes from blue to purple in color. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 2.431 mg of Mg.

**Containers and storage** Containers—Tight containers.

---

**Magnesium Sulfate Hydrate**

硫酸マグネシウム水和物

\[
\text{MgSO}_4\cdot\text{7H}_2\text{O} : 246.47
\]

Magnesium Sulfate Hydrate, when ignited, contains not less than 99.0% of magnesium sulfate (\( \text{MgSO}_4 \cdot 120.37 \)).

**Description** Magnesium Sulfate Hydrate occurs as colorless or white crystals. It has a cooling, saline, bitter taste. It is very soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

**Identification** A solution of Magnesium Sulfate Hydrate (1 in 40) responds to the Qualitative Tests for magnesium salt and for sulfate.

\[
\text{pH} < 2.54 \quad \text{Dissolve 1.0 g of Magnesium Sulfate Hydrate in 20 mL of water: the pH of this solution is between 5.0 and 8.2.}
\]

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Magnesium Sulfate Hydrate in 20 mL of water: the solution is clear and colorless.

(2) Chloride

\[
\text{Cl} < 1.05 \quad \text{Perform the test with 1.0 g of Magnesium Sulfate Hydrate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).}
\]

(3) Heavy metals

\[
\text{< 1.077 } \quad \text{Proceed with 2.0 g of Magnesium Sulfate Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).}
\]

(4) Zinc

\[
\text{< 2.0 g of Magnesium Sulfate Hydrate in 20 mL of water, and add 1 mL of acetic acid and 5 drops of potassium hexacyanoferrate (II) TS: no turbidity is produced.}
\]
(5) Calcium—Dissolve 1.0 g of Magnesium Sulfate Hydrate in 5.0 mL of dilute hydrochloric acid, add water to make 100 mL, and use this solution as the sample solution. Separately, dissolve 1.0 g of Magnesium Sulfate Hydrate in 2.0 mL of Standard Calcium Solution and 5.0 mL of dilute hydrochloric acid, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions, and determine the absorbances, \( A_T \) and \( A_S \), of both solutions: \( A_T \) is not bigger than \( A_S - A_T \) (not more than 0.02%).

Gas: Combustible gas—Acetylene or hydrogen.
Supporting gas—Air.
Lamp: Calcium hollow-cathod lamp.
Wavelength: 422.7 nm.

(6) Arsenic <1.17>—Prepare the test solution with 1.0 g of Magnesium Sulfate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

Loss on ignition <2.43> 45.0 – 52.0% (1 g, after drying at 105°C for 2 hours, ignite at 450°C for 3 hours).

Assay Weigh accurately about 0.6 g of Magnesium Sulfate Hydrate, previously ignited at 450°C for 3 hours after drying at 105°C for 2 hours, and dissolve in 2 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 25 mL of this solution, add 50 mL of water and 5 mL of ammonium-chloride buffer solution, \( \text{pH} \approx 10.7 \), and titrate <2.50> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination, and make any necessary correction.

\[
\text{Each mL of} \ 0.05 \text{ mol/L disodium dihydrogen ethylenediamine tetraacetate VS} = 6.018 \text{ mg of MgSO}_4
\]

Containers and storage Containers—Well-closed containers.

Magnesium Sulfate Injection
硫酸マグネシウム水

Magnesium Sulfate Injection is an aqueous solution for injection.
It contains not less than 95.0% and not more than 105.0% of the labeled amount of magnesium sulfate hydrate (\( \text{MgSO}_4 \cdot 7\text{H}_2\text{O} \): 246.47).

Method of preparation Prepare as directed under Injections, with Magnesium Sulfate Hydrate.

Description Magnesium Sulfate Injection is a clear, colorless liquid.

Identification Measure a volume of Magnesium Sulfate Injection, equivalent to 0.5 g of Magnesium Sulfate Hydrate according to the labeled amount, and add water to make 20 mL: the solution responds to the Qualitative Tests <1.09> for magnesium salt and for sulfate.

\( \text{pH} <2.54 \) 5.5 – 7.0 When the labeled concentration exceeds 5%, prepare a solution of 5% with water, and perform the test.

Bacterial endotoxins <4.07> Less than 0.09 EU/mg.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Magnesium Sulfate Injection, equivalent to about 0.3 g of magnesium sulfate hydrate (\( \text{MgSO}_4 \cdot 7\text{H}_2\text{O} \)), and add water to make 75 mL. Then add 5 mL of ammonia-ammonium chloride buffer solution, \( \text{pH} \approx 10.7 \), and proceed as directed in the Assay under Magnesium Sulfate Hydrate.

\[
\text{Each mL of} \ 0.05 \text{ mol/L disodium dihydrogen ethylenediamine tetraacetate VS} = 12.32 \text{ mg of MgSO}_4 \cdot 7\text{H}_2\text{O}
\]

Containers and storage Containers—Tight containers.

Magnesium Sulfate Mixture
硫酸マグネシウム注射液

Magnesium Sulfate Mixture contains not less than 13.5 w/v% and not more than 16.5 w/v% of magnesium sulfate hydrate (\( \text{MgSO}_4 \cdot 7\text{H}_2\text{O} \): 246.47).

Method of preparation

Prepare before use, with the above ingredients.

Description Magnesium Sulfate Mixture is a light yellowish clear liquid. It has a bitter and acid taste.

Identification (1) Magnesium Sulfate Mixture responds to the Qualitative Tests <1.09> for magnesium salt.
(2) Magnesium Sulfate Mixture responds to the Qualitative Tests <1.09> (2) for chloride.

Assay Pipet 10 mL of Magnesium Sulfate Mixture, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add 50 mL of water and 5 mL of \( \text{pH} \approx 10.7 \) ammonium-chloride buffer solution, and titrate <2.50> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator).

\[
\text{Each mL of} \ 0.05 \text{ mol/L disodium dihydrogen ethylenediamine tetraacetate VS} = 12.32 \text{ mg of MgSO}_4 \cdot 7\text{H}_2\text{O}
\]

Containers and storage Containers—Tight containers.
Maltose Hydrate

マルトース水和物

\[ \text{C}_{12}\text{H}_{22}\text{O}_{11} \cdot \text{H}_{2}\text{O} \] = 360.31

[\alpha\text{-d-Glucopyranosyl-(1→4)-}\beta\text{-d-gluco}pyranose monohydrate]

[Maltose Hydrate, when dried, contains not less than 98.0% of \( \text{C}_{12}\text{H}_{22}\text{O}_{11} \cdot \text{H}_{2}\text{O} \).]

**Description**

Maltose Hydrate occurs as white crystals or crystalline powder.

It has a sweet taste.

It is freely soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

**Identification**

1. Dissolve 0.5 g of Maltose Hydrate in 5 mL of water, add 5 mL of ammonia TS, and heat for 5 minutes on a water bath: an orange color develops.

2. Add 2 to 3 drops of a solution of Maltose Hydrate (1 in 50) to 5 mL of boiling Fehling TS: a red precipitate is formed.

**Optical rotation**

\[ (\alpha)_D^2 = +126 \to +131^\circ \]

Weigh accurately about 10 g of Maltose Hydrate, previously dried, dissolve in 0.2 mL of ammonia TS and water to make exactly 100 mL, and determine the optical rotation of this solution in a 100-mm cell.

**pH**

\[ < 2.55 \]

The pH of a solution of Maltose Hydrate (1 in 10) is between 4.5 and 6.5.

**Purity**

1. Clarity and color of solution—Put 10 g of Maltose Hydrate in 30 mL of water in a Nessler tube, warm at 60°C in a water bath to dissolve, and after cooling, add water to make 50 mL: the solution is clear, and has no more color than the following control solution.

   Control solution: Add water to a mixture of 1.0 mL of Cobalt (II) Chloride CS, 3.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS to make 10.0 mL. To 1.0 mL of this solution add water to make 50 mL.

2. Chloride

   Perform the test with 2.0 g of Maltose Hydrate. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

3. Sulfate

   Perform the test with 2.0 g of Maltose Hydrate. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

4. Heavy metals

   Proceed with 5.0 g of Maltose Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 4 ppm).

5. Arsenic

   Dissolve 1.5 g of Maltose Hydrate in 5 mL of water, add 5 mL of dilute sulfuric acid and 1 mL of bromine TS, heat on a water bath for 5 minutes, then heat to concentrate to 5 mL, and use this solution as the test solution after cooling. Perform the test (not more than 1.3 ppm).

6. Dextrin, soluble starch and sulfite

   Dissolve 1.0 g of Maltose Hydrate in 10 mL of water, and add 1 drop of iodine TS: a yellow color appears, and the color changes to a blue by adding 1 drop of starch TS.

7. Nitrogen

   Weigh accurately about 2 g of Maltose Hydrate, and perform the test as directed under Nitrogen Determination using 10 mL of sulfuric acid for the decomposition and 45 mL of a solution of sodium hydroxide (2 in 5) for the addition: the amount of nitrogen (N: 14.01) is not more than 0.01%.

8. Related substances

   Dissolve 0.5 g of Maltose Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine the peak areas from both solutions by the automatic integration method: the total area of the peaks which appear before the peak of maltose from the sample solution is not larger than 1.5 times the peak area of maltose from the standard solution, and the total area of the peaks which appear after the peak of maltose from the sample solution is not larger than 1/2 times the peak area of maltose from the standard solution.

   **Operating conditions**

   Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed as directed in the operating conditions in the Assay.

   Detection sensitivity: Adjust the sensitivity so that the peak height of maltose obtained from 20 μL of the standard solution is about 30 mm.

   Time span of measurement: About 2 times as long as the retention time of maltose.

**Loss on drying**

\[ < 0.5\% \]

(1 g, 80°C, 4 hours).

**Residue on ignition**

\[ < 0.1\% \]

(1 g).

**Assay**

Weigh accurately about 0.1 g each of Maltose Hydrate and Maltose RS, previously dried, dissolve in exactly 10 mL each of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following operating conditions, and calculate the ratios, \( Q_1 \) and \( Q_2 \), of the peak area of maltose to that of the internal standard.

\[
\text{Amount (mg) of } \text{C}_{12}\text{H}_{22}\text{O}_{11} \cdot \text{H}_{2}\text{O} = M_S \times \frac{Q_1}{Q_2}
\]

\( M_S \): Amount (mg) of Maltose RS

**Internal standard solution**

A solution of ethylene glycol (1 in 50).

**Operating conditions**

Detector: A differential refractometer.

Column: A stainless steel column about 8 mm in inside diameter and about 55 cm in length, packed with gel-type strong acid cation-exchange resin for liquid chromatography (degree of cross-linking: 8%) (10 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Water.

Flow rate: Adjust the flow rate so that the retention time

...
of maltose is about 18 minutes.

Selection of column: Dissolve 0.25 g of maltose, 0.25 g of glucose and 0.4 g of ethylene glycol in water to make 100 mL. Proceed with 20 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of maltose, glucose and ethylene glycol in this order with the resolution of between the peaks of maltose and glucose being not less than 4.

Containers and storage Containers—Tight containers.

Freeze-dried Mamushi Antivenom, Equine

乾燥まむしウマ抗毒素

Freeze-dried Mamushi Antivenom, Equine, is a preparation for injection which is dissolved before use. It contains Agkistrodon Halys antivenin in immunoglobulin of horse origin. It conforms to the requirements of Freeze-dried Mamushi Antivenom, Equine, in the Minimum Requirements for Biological Products.

Description Freeze-dried Mamushi Antivenom, Equine, becomes a colorless or light yellow-brown, clear liquid, or a slightly white-turbid liquid on addition of solvent.

Manidipine Hydrochloride

マンジピン塩酸塩

\[
\text{C}_{35}\text{H}_{38}\text{N}_{4}\text{O}_{6}\cdot 2\text{HCl} \quad \text{683.62}
\]

3-(2-[4-(2-phenyl methyl)piperazin-1-yl]ethyl)-5-methyl (4RS)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate dihydrochloride [126229-12-7]

Manidipine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of C_{35}H_{38}N_{4}O_{6}.2HCl.

Description Manidipine Hydrochloride occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in dimethylsulfoxide, sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Manidipine Hydrochloride in dimethylsulfoxide (1 in 100) shows no optical rotation.

Manidipine Hydrochloride turns slightly brown-yellowish white on exposure to light.

Melting point: about 207°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Manidipine Hydrochloride in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Manidipine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Manidipine Hydrochloride as directed in the potassium chloride disc method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Manidipine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Add 10 mL of water to 0.1 g of Manidipine Hydrochloride, shake vigorously, and filter. Add 1 drop of ammonium TS to 3 mL of the filtrate, allow to stand 5 minutes, and filter. The filtrate responds to the Qualitative Tests <1.09> (2) for chlorides.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Manidipine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 2.0 g of Manidipine Hydrochloride according to Method 4, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 20 mg of Manidipine Hydrochloride in 200 mL of a mixture of water and acetonitrile (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and acetonitrile (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area from both solutions by the automatic integration method: the area of the peaks other than manidipine obtained from the sample solution is not larger than 1/5 times the manidipine peak area from the standard solution. Furthermore, the total of the areas of all peaks other than manidipine is not larger than 7/10 times the peak area of manidipine from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3.5 times as long as the retention time of manidipine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 10 mL of the standard solution, add a mixture of water and acetonitrile (1:1) to make exactly 100 mL. Confirm that the peak area of manidipine obtained from 20 μL of this solution is equivalent to 8 to 12% of that from 20 μL of the standard solution.

System performance: Dissolve 50 mg of Manidipine Hydrochloride in a mixture of water and acetonitrile (1:1) to make 50 mL. To 10 mL of this solution add 5 mL of a solution of butyl benzoate in acetonitrile (7 in 5000) and the mixture of water and acetonitrile (1:1) to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, manidipine and butyl benzoate...
are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of manidipine is not more than 2.0%.

**Loss on drying <2.4%** Not more than 1.5% (1 g, 105°C, 4 hours).

**Residue on ignition <2.4%** Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.1 g of Manidipine Hydrochloride, previously dried, and dissolve in a mixture of water and acetonitrile (1:1) to make exactly 50 mL. Pipet 20 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, dissolve 10 mg of Manidipine Hydrochloride Tablets, equivalent to 10 mg of Manidipine Hydrochloride according to the labeled amount, add 5 mL of methanol, shake vigorously, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of Manidipine Hydrochloride RS in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.02> to that of the internal standard. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_2 \), of the peak area of manidipine to that of the internal standard.

\[
\text{Amount (mg) of } C_{35}H_{38}N_4O_6\cdot2\text{HCl} = M_5 \times Q_1/Q_2 \times 4
\]

\( M_5 \): Amount (mg) of Manidipine Hydrochloride RS

**Internal standard solution—A solution of butyl benzoate in acetonitrile (7 in 5000).**

**Operating conditions—**
- Detector: An ultraviolet absorption photometer (wavelength: 228 nm).
- Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 25°C.
- Mobile phase: Dissolve 13.6 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 4.6 with diluted potassium hydroxide TS (1 in 10). To 490 mL of this solution add 510 mL of acetonitrile.
- Flow rate: Adjust the flow rate so that the retention time of manidipine is about 10 minutes.

**System suitability—**
- System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, manidipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.
- System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of manidipine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.
- Storage—Light-resistant.

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**Manidipine Hydrochloride Tablets**

Manidipine Hydrochloride Tablets contain not less than 92.0% and not more than 108.0% of the labeled amount of manidipine hydrochloride (\( C_{35}H_{38}N_4O_6\cdot2\text{HCl} \): 683.62).

**Method of preparation** Prepare as directed under Tablets, with Manidipine Hydrochloride.

**Identification** To a quantity of powdered Manidipine Hydrochloride Tablets, equivalent to 10 mg of Manidipine Hydrochloride according to the labeled amount, add 5 mL of methanol, shake vigorously, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of Manidipine Hydrochloride RS in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.02>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and diethylamine (200:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot obtained from the standard solution show the same \( R_f \) value.

**Uniformity of dosage units <6.02>** Perform the test according to the following method: it meets the requirement of the Content uniformity test. Conduct this procedure using light-resistant vessels.

Conduct this procedure using light-resistant vessels. To 1 tablet of Manidipine Hydrochloride Tablets, add exactly 1 mL of the internal standard solution per 1 mg of manidipine hydrochloride (\( C_{35}H_{38}N_4O_6\cdot2\text{HCl} \)), disintegrate by adding a mixture of water and acetonitrile (1:1) to make \( V \) mL so that each mL contains about 0.1 mg of manidipine hydrochloride (\( C_{35}H_{38}N_4O_6\cdot2\text{HCl} \)), shake vigorously for 10 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed in the Assay.

\[
\text{Amount (mg) of manidipine hydrochloride} = M_5 \times Q_1/Q_2 \times V/250
\]

\( M_5 \): Amount (mg) of Manidipine Hydrochloride RS

**Internal standard solution—A solution of butyl benzoate in acetonitrile (7 in 10,000).**

**Dissolution <6.10>** When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, as the dissolution medium, the dissolution rate in 45 minutes of Manidipine Hydrochloride Tablets is not less than 75%.

Conduct this procedure using light-resistant vessels. Start the test with 1 tablet of Manidipine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the
first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, and add the dissolution medium to make exactly V mL so that each mL contains about 5.6 μg of manidipine hydrochloride \((C_{35}H_{38}N_{4}O_{6} \cdot 2HCl)\) according to the labeled amount. Pipet 2 mL of this solution, add exactly 2 mL of methanol, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Manidipine Hydrochloride RS, previously dried, dissolve in a mixture of water and acetonitrile (1:1) to make exactly 50 mL. Pipet 1 mL of this solution, and add the dissolution medium to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of methanol, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\) according to the following conditions, and determine the manidipine peak areas, \(A_T\) and \(A_S\), of both solutions.

Dissolution rate (%) with respect to the labeled amount of manidipine hydrochloride \((C_{35}H_{38}N_{4}O_{6} \cdot 2HCl)\)

\[ M_S: \text{Amount (mg) of Manidipine Hydrochloride RS} \]

\[ C: \text{Labeled amount (mg) of manidipine hydrochloride } (C_{35}H_{38}N_{4}O_{6} \cdot 2HCl) \text{ in 1 tablet} \]

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 228 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile and a solution of potassium dihydrogen phosphate (681 in 100,000) (3:2).

Flow rate: Adjust the flow rate so that the retention time of manidipine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of manidipine are not less than 1500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of manidipine is not more than 2.0%.

Assay

Conduct this procedure using light-resistant vessels. Weigh accurately not less than 20 Manidipine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of manidipine hydrochloride \((C_{35}H_{38}N_{4}O_{6} \cdot 2HCl)\), add exactly 10 mL of the internal standard solution, add a mixture of water and acetonitrile (1:1) to make 100 mL, shake vigorously for 10 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Manidipine Hydrochloride RS, previously dried, and dissolve in the mixture of water and acetonitrile (1:1) to make 50 mL. Pipet 20 mL of this solution, add exactly 10 mL of the internal standard solution, add the mixture of water and acetonitrile (1:1) to make 100 mL, and use this solution as the standard solution.

Then, proceed as directed in the Assay under Manidipine Hydrochloride.

\[ \text{Amount (mg) of manidipine hydrochloride} \]

\[ (C_{35}H_{38}N_{4}O_{6} \cdot 2HCl) = M_S \times Q_T/ Q_S \times 2/5 \]

\( M_S: \text{Amount (mg) of Manidipine Hydrochloride RS} \)

Internal standard solution—A solution of butyl benzoate in acetonitrile (7 in 10,000).

Containers and storage—Containers—Tight containers. Storage—Light-resistant.

**d-Mannitol**

\(\text{d-マンニトール}\)

\(\text{C}_{6}\text{H}_{14}\text{O}_{6}: 182.17\)

\(\text{d-Mannitol} [69-65-8]\)

\(\text{d-Mannitol, when dried, contains not less than 98.0\% of C}_{6}\text{H}_{14}\text{O}_{6}.\)

**Description**

D-Mannitol occurs as white crystals or powder. It is odorless, and has a sweet taste with a cold sensation.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It dissolves in sodium hydroxide TS.

**Identification (1)**

To 5 drops of a saturated solution of \(\text{d-Mannitol}\) add 1 mL of iron (III) chloride TS and 5 drops of a solution of sodium hydroxide (1 in 5): a yellow precipitate is produced. Add 1 mL of ferric chloride TS and 5 drops of a solution of sodium hydroxide (1 in 5): a yellow precipitate is produced. On addition of a solution of sodium hydroxide (1 in 5), no precipitate is produced.

**Optical rotation** \(<2.49\>\quad [\alpha]_{D}^{D}: +137 – +145^\circ\)

Weigh accurately about 1.0 g of \(\text{d-Mannitol}\), previously dried, dissolve in 80 mL of a solution of hexaammonium heptamolybdate tetrahydrate (1 in 20), and add diluted sulfuric acid (1 in 35) to make exactly 100 mL. Measure the optical rotation of this solution in a 100-mm cell.

**Melting point** \(<2.60\>\quad 166 – 169°C\)

**Purity** (1) Clarity and color of solution—Dissolve 2.0 g of \(\text{d-Mannitol}\) in 10 mL of water by warming: the solution is clear and colorless.
Acidity—Dissolve 5.0 g of d-Mannitol in 50 mL of freshly boiled and cooled water, and add 1 drop of phenolphthalein TS and 0.50 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

Chloride
Perform the test with 2.0 g of d-Mannitol. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.007%).

Sulfate
Perform the test with 2.0 g of d-Mannitol. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

Heavy metals
Proceed with 5.0 g of d-Mannitol according to Method 1, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

Nickel—Dissolve 0.5 g of d-Mannitol in 5 mL of water, add 3 drops of dimethylglyoxime TS and 3 drops of ammonia TS, and allow to stand for 5 minutes: no red color develops.

Arsenic
Prepare the test solution with 1.5 g of d-Mannitol according to Method 1, and perform the test (not more than 1.3 ppm).

Sugars—To 5.0 g of d-Mannitol add 15 mL of water and 4.0 mL of dilute hydrochloric acid, and heat under a reflux condenser in a water bath for 3 hours. After cooling, neutralize with sodium hydroxide TS (indicator: 2 drops of methyl orange TS), and add water to make 50 mL. Pipet 10 mL of this solution into a flask, boil gently with 10 mL of water and 40 mL of Fehling’s TS for 3 minutes, and allow to stand to precipitate copper (I) oxide. Filter the supernatant liquid through a glass filter (G4), wash the precipitate with hot water until the last washing no longer shows an alkaline reaction, and filter the washings through the glass filter described above. Dissolve the precipitate in 20 mL of iron (III) sulfate TS in the flask, filter through the glass filter described above, and wash the filter with water. Combine the washings and the filtrate, heat to 80°C, and titrate with 0.02 mol/L potassium permanganate VS: the consumed volume is not more than 1.0 mL.

Loss on drying
Not more than 0.30% (1 g, 105°C, 4 hours).

Residue on ignition
Not more than 0.1% (1 g).

Assay
Weigh accurately about 0.2 g of d-Mannitol, previously dried, and dissolve in water to make exactly 100 mL. Pipet 10 mL of the solution into an iodine flask, add exactly 50 mL of potassium periodate TS, and heat for 15 minutes in a water bath. After cooling, add 2.5 g of potassium iodide, stopper tightly, and shake well. Allow to stand for 5 minutes in a dark place, and titrate with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium thiosulfate VS = 1.822 mg of C$_6$H$_{14}$O$_6$.

Containers and storage
Containers—Tight containers.

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**D-Mannitol Injection**

**p-Mannite Injection**

d-マンニトール注射液

D-Mannitol Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of d-mannitol (C$_6$H$_{14}$O$_6$: 182.17).

**Method of preparation**
Prepare as directed under Injections, with d-Mannitol. No preservative is added.

**Description**
D-Mannitol Injection is a clear, colorless liquid. It has a sweet taste.

It may precipitate crystals.

**Identification**
Concentrate D-Mannitol Injection on a water bath to make an saturated solution. Proceed with 5 drops of this solution as directed in the Identification (1) under d-Mannitol.

**pH**
4.5 – 7.0

**Bacterial endotoxins**
Less than 0.50 EU/mL.

**Extractable volume**
It meets the requirement.

**Foreign insoluble matter**
Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter**
It meets the requirement.

**Sterility**
Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay**
Measure exactly a volume of d-Mannitol Injection, equivalent to about 5 g of d-mannitol (C$_6$H$_{14}$O$_6$), and add water to make exactly 250 mL. To exactly 10 mL of this solution add water to make exactly 100 mL. Measure exactly 10 mL of this solution into an iodine flask, and proceed as directed in the Assay under d-Mannitol.

Each mL of 0.1 mol/L sodium thiosulfate VS = 1.822 mg of C$_6$H$_{14}$O$_6$.

Containers and storage
Containers—Hermetic containers.
Plastic containers for aqueous injections may be used.
Maprotiline Hydrochloride

マプロチリン塩酸塩

C₂₀H₂₃N.HCl: 313.86
3-(9,10-Dihydro-9,10-ethanoanthracen-9-yl)-N-methylpropylamine monohydrochloride [10347-81-6]

Maprotiline Hydrochloride, when dried, contains not less than 99.0% of C₂₀H₂₃N.HCl.

Description Maprotiline Hydrochloride occurs as a white crystalline powder.
It is soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (99.5), and slightly soluble in water.
Melting point: about 244°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Maprotiline Hydrochloride in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Maprotiline Hydrochloride, previously dried, as directed in Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize Maprotiline Hydrochloride with ethanol (99.5), filter, dry the crystals so obtained, and perform the test with the crystals.

(3) To 5 mL of a solution of Maprotiline Hydrochloride (1 in 200) add 2 mL of ammonia TS, heat on a water bath for 5 minutes, cool, and filter. Acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Maprotiline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.10 g of Maprotiline Hydrochloride in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 μL of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop with a mixture of 2-butanol, diluted ammonia solution (28) (1 in 3) and ethyl acetate (14:5:4) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spot other than the principal spot from the sample solution is not more than 2 and they are not more intense than the spot from the standard solution.

Assay Weigh accurately about 0.25 g of Maprotiline Hydrochloride, previously dried, dissolve in 180 mL of acetic acid (100), add 8 mL of a solution of bismuth nitrate pentahydrate in acetic acid (100) (1 in 50), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 31.39 mg of C₂₀H₂₃N.HCl

Containers and storage Containers—Well-closed containers.

Freeze-dried Live Attenuated Measles Vaccine

乾燥弱毒生麻しんワクチン

Freeze-dried Live Attenuated Measles Vaccine is a preparation for injection which is dissolved before use. It contains live attenuated measles virus. It conforms to the requirements of Freeze-dried Live Attenuated Measles Vaccine in the Minimum Requirements for Biological Products.

Description Freeze-dried Live Attenuated Measles Vaccine becomes a colorless, yellowish or reddish clear liquid on addition of solvent.

Meclofenoxate Hydrochloride

メクロフェノキサート塩酸塩

C₁₂H₁₆ClNO₃.HCl: 294.17
2-(Dimethylamino)ethyl (4-chlorophenoxy)acetate monohydrochloride [3685-84-5]

Meclofenoxate Hydrochloride contains not less than 98.0% of C₁₂H₁₆ClNO₃.HCl, calculated on the anhydrous basis.

Description Meclofenoxate Hydrochloride occurs as white crystals or crystalline powder. It has a faint, characteristic odor and a bitter taste.
It is freely soluble in water and in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.
The pH of a solution of Meclofenoxate Hydrochloride (1 in 20) is between 3.5 and 4.5.

Identification (1) To 10 mg of Meclofenoxate Hydrochlo-
Mecobalamin / Official Monographs

Mecobalamin

**Mecobalamin**

![Mecobalamin molecular structure]

\[
C_{63}H_{91}CoN_{13}O_{14}P: 1344.38 \\
Co-a-(5,6-Dimethyl-1H-benzoimidazol-1-yl)-Cofb-methylcobamide \\
[13422-35-4]
\]

Mecobalamin contains not less than 98.0% of \(C_{63}H_{91}CoN_{13}O_{14}P\), calculated on the anhydrous basis.

**Description** Mecobalamin occurs as dark red crystals or crystalline powder.

It is sparingly soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in acetonitrile.

It is affected by light.

**Identification** (1) Conduct this procedure without exposure to light, using light-resistant vessels. Determine the absorption spectrum of a solution of Mecobalamin in hydrochloric acid-potassium chloride buffer solution, pH 2.0 (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1 or the spectrum of a solution of Mecobalamin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Mecobalamin in phosphate buffer solution, pH 7.0 (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2 or the spectrum of a solution of Mecobalamin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Mix 1 mg of Mecobalamin with 50 mg of potassium bisulfate, and fuse by igniting. Cool, break up the mass with a glass rod, add 3 mL of water, and dissolve by boiling. Add 1 drop of phenolphthalein TS, then add dropwise sodium hydroxide TS until a light red color just develops. Add 0.5 g of sodium acetate, 0.5 mL of dilute acetic acid and 0.5 mL of a solution of disodium 1-nitroso-2-naphthol-3,6-disulfonate (1 in 500): a red to orange-red color is immediately produced. Then add 0.5 mL of hydrochloric acid, and boil for 1 minute: the red color does not disappear.

**Ride** add 2 mL of ethanol (95), dissolve by warming if necessary, cool, add 2 drops of a saturated solution of hydroxylammonium chloride in ethanol (95) and 2 drops of a saturated solution of potassium hydroxide in ethanol (95), and heat in a water bath for 2 minutes. After cooling, render the solution slightly acidic with dilute hydrochloric acid, and add 3 drops of iron (II) chloride TS: a red-purple to dark purple color develops.

(2) Dissolve 50 mg of Meclofenoxate Hydrochloride in 5 mL of water, and add 2 drops of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the absorption spectrum of a solution of Meclofenoxate Hydrochloride (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution of Meclofenoxate Hydrochloride (1 in 100) responds to the Qualitative Tests <1.89> for chloride.

**Melting point** <2.68> 139 – 143°C

**Purity** (1) Clarify and color of solution—Dissolve 0.5 g of Meclofenoxate Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 1.0 g of Meclofenoxate Hydrochloride. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Meclofenoxate Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.12>—Prepare the test solution with 1.0 g of Meclofenoxate Hydrochloride according to method 3, and perform the test (not more than 2 ppm).

(5) Organic acids—To 2.0 g of Meclofenoxate Hydrochloride add 50 mL of diethyl ether, shake for 10 minutes, filter through a glass filter (G3), wash the residue with two 5-mL portions of diethyl ether, and combine the washings with the filtrate. To this solution add 50 mL of neutralized ethanol and 5 drops of phenolphthalein TS, and neutralize with 0.1 mol/L sodium hydroxide VS: the volume of 0.1 mol/L sodium hydroxide VS consumed is not more than 0.54 mL.

**Water** <2.48> Not more than 0.50% (1 g, volumetric titration, direct titration).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Meclofenoxate Hydrochloride, dissolve in 70 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from blue-green through yellow-green to pale greenish yellow [indicator: 3 drops of a solution of malachite green oxalate in acetic acid (1 in 1000) in a water bath for 2 minutes. After cooling, render the solution slightly acidic with dilute hydrochloric acid, and add 3 drops of iron (II) chloride TS: a red-purple to dark purple color develops.

\[
\text{Each mL of 0.1 mol/L perchloric acid VS} = 29.42 \text{mg of } C_{12}H_{8}ClCNO_3\cdot HCl
\]

**Containers and storage** Containers—Tight containers.
**Purity** (1) Clarity and color of solution—Dissolve 20 mg of Mecobalamin in 10 mL of water: the solution is clear and red color.

(2) Related substances—Perform the test with 10 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography 〈2.01〉 according to the following conditions. Determine the peak area of Mecobalamin and others of the sample solution by the automatic integration method: each area of the peaks other than Mecobalamin is not more than 0.5% of the peak area of Mecobalamin, and the total area of the peaks other than Mecobalamin is not more than 2.0%.

**Operating conditions—**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of Mecobalamin.

**System suitability—**

Test for required detection: To exactly 1 mL of the sample solution add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add the mobile phase to make exactly 10 mL. Confirm that the peak area of Mecobalamin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak areas of Mecobalamin is not more than 3.0%.

**Water** 〈2.48〉 Not more than 12% (0.1 g, volumetric titration, direct titration).

**Assay** Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately about 50 mg of Mecobalamin and Mecobalamin RS (separately, determine the water 〈2.48〉 in the same manner as Mecobalamin), dissolve each in the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography 〈2.01〉 according to the following conditions, and determine the peak areas, A₁ and A₅, of Mecobalamin in each solution.

\[
\text{Amount (mg) of } C_{63}H_{91}CoN_{13}O_{14}P = M_5 \times \frac{A_1}{A_5}
\]

\[
M_5: \text{Amount (mg) of Mecobalamin RS, calculated on the anhydrous basis}
\]

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 266 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 200 mL of acetonitrile add 800 mL of 0.02 mol/L phosphate buffer solution, pH 3.5, then add 3.76 g of sodium 1-hexane sulfonate to dissolve.

Flow rate: Adjust the flow rate so that the retention time of Mecobalamin is about 12 minutes.

**System suitability—**

System performance: Dissolve 5 mg each of cyanocobalamin and hydroxocobalamin acetate in the mobile phase to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, cyanocobalamin and hydroxocobalamin are eluted in this order with the resolution between these peaks being not less than 3. And when the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of Mecobalamin is not less than 6000.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of Mecobalamin is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

**Medazepam**

メダゼパム

**Identification** (1) Dissolve 10 mg of Medazepam in 3 mL of citric acid-acetic acid TS: a deep orange color develops. Heat in a water bath for 3 minutes: the color changes to dark red.

(2) Determine the absorption spectrum of a solution of Medazepam in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 〈2.24〉, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Medazepam as directed in the potassium bromide disk method under Infrared Spectrophotometry 〈2.25〉, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave length.
Perform the test with Medazepam as directed under Flame Coloration Test \(<1.04\rangle\) (2): a green color appears.

**Melting point** \(<2.60\rangle\quad 101 - 104^\circ C\)

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Medazepam in 10 mL of methanol: the solution is clear and light yellow to yellow in color.

(2) Chloride \(<1.03\rangle\)—Dissolve 1.5 g of Medazepam in 50 mL of diethyl ether, add 46 mL of water and 4 mL of sodium carbonate TS, shake, and collect the water layer. Wash the water layer with two 20-mL portions of diethyl ether, and filter. To 20 mL of the filtrate add dilute nitric acid to neutralize, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(3) Heavy metals \(<1.07\rangle\)—Proceed with 1.0 g of Medazepam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic \(<1.11\rangle\)—Prepare the test solution with 1.0 g of Medazepam according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.25 g of Medazepam in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 2 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.60\rangle\). Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, acetone and ammonia solution (28) (60:40:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** \(<2.41\rangle\quad \text{Not more than 0.5} \% \quad \text{(1 g, in vacuum, 60 } ^\circ C, 4 \text{ hours)}\).

**Residue on ignition** \(<2.44\rangle\quad \text{Not more than 0.1} \% \quad \text{(1 g)}\).

**Assay** Weigh accurately about 0.4 g of Medazepam, previously dried, dissolve in 50 mL of acetic acid (100), and titrate \(<2.50\rangle\) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

\[
\text{Each mL of 0.1 mol/L perchloric acid VS} = 27.08 \text{ mg of C}_{16}H_{15}ClN_{2}
\]

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

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**Medicinal Carbon**

**Description** Medicinal Carbon occurs as a black, odorless and tasteless powder.

**Identification** Place 0.5 g of Medicinal Carbon in a test tube, and heat by direct application of flame with the aid of a current of air: it burns without any flame. Pass the evolved gas through calcium hydroxide TS: a white turbidity is produced.

**Purity** (1) Acidity or alkalinity—Boil 3.0 g of Medicinal Carbon with 60 mL of water for 5 minutes, allow to cool, dilute to 60 mL with water, and filter: the filtrate is colorless and neutral.

(2) Chloride \(<1.03\rangle\)—Take 4.0 mL of the filtrate obtained in (1) in a Nessler tube, add 6 mL of dilute nitric acid and sufficient water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.80 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.142%).

(3) Sulfate \(<1.14\rangle\)—Take 5 mL of the filtrate obtained in (1) in a Nessler tube, and add 1 mL of dilute hydrochloric acid and sufficient water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.192%).

(4) Sulfide—Boil 0.5 g of Medicinal Carbon with a mixture of 15 mL of dilute hydrochloric acid and 10 mL of water: lead (II) acetate paper does not become brown when held in the evolved gas within 5 minutes.

(5) Cyanogen compounds—Place a mixture of 5 g of Medicinal Carbon, 2 g of l-tartaric acid and 50 mL of water in a distilling flask connected to a condenser provided with a tightly fitting adapter, the end of which dips below the surface of a mixture of 2 mL of sodium hydroxide TS and 10 mL of water, contained in a small flask surrounded by ice. Heat the mixture in the distilling flask to boiling, and distill to 25 mL. Dilute the distillate with water to 50 mL. To 25 mL of the diluted distillate add 1 mL of a solution of iron (II) sulfate heptahydrate (1 in 20), heat the mixture almost to boiling, cool, and filter. To the filtrate add 1 mL of hydrochloric acid and 0.5 mL of dilute iron (III) chloride TS: no blue color is produced.

(6) Acid soluble substances—To about 1 g of Medicinal Carbon, accurately weighed, add 20 mL of water and 5 mL of hydrochloric acid, boil for 5 minutes, filter, wash the residue with 10 mL of hot water, and add the washings to the filtrate. Add 5 drops of sulfuric acid to the filtrate, evaporate to dryness, and ignite the residue strongly: the mass of the residue is not more than 3.0%.

(7) Heavy metals \(<1.07\rangle\)—Proceed with 0.5 g of Medicinal Carbon according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 50 ppm).

(8) Zinc—Ignite 0.5 g of Medicinal Carbon to ash, add 5 mL of dilute nitric acid to the residue, boil gently for 5 minutes, filter, wash with 10 mL of water, and combine the washings and the filtrate. Add 3 mL of ammonia TS to the solution, filter again, wash with water, combine the washings and the filtrate, add another washing to make 25 mL,
add 1 drop of sodium sulfide TS, and allow to stand for 3 minutes: the liquid produces no turbidity.

(9) Arsenic \(<1.11\) — Prepare the test solution with 1.0 g of Medicinal Carbon according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying \(<2.41\) — Not more than 15.0% (1 g, 105°C, 4 hours).

Residue on ignition \(<2.44\) — Not more than 4.0% (1 g).

Adsorptive power (1) Add 1.0 g of Medicinal Carbon, previously dried, to 100 mL of water containing 120 mg of quinine sulfate, shake the mixture vigorously for 5 minutes, filter immediately, and reject the first 20 mL of the filtrate. Add 5 drops of iodine TS to 10 mL of the subsequent filtrate: no turbidity is produced.

(2) Dissolve 250 mg of methylene blue trihydrate, exactly weighed, in water to make exactly 250 mL. Measure two 50-mL portions of this solution into each of two glass-stoppered flasks. To one flask add exactly 250 mg of Medicinal Carbon, previously dried, and shake vigorously for 5 minutes. Filter the contents of each flask, rejecting the first 20 mL of each filtrate. Add 25 mL portions of the remaining filtrate into two 250-mL volumetric flasks. To each volumetric flask add 50 mL of a solution of sodium acetate trihydrate (1 in 10), then add exactly 35 mL of 0.05 mol/L iodine solution (not more than 20 ppm).

(3) Ethanol-insoluble substances — Weigh accurately about 2 g of Medicinal Soap, dissolve by warming in 100 mL of neutralized ethanol, filter the solution through a glass filter (G4), wash the residue with hot neutralized ethanol, and dry at 105°C for 4 hours: the mass of the residue is not more than 1.0%.

Water-insoluble substances — Wash thoroughly the dried substances obtained in (3) with 200 mL of water, and dry at 105°C for 4 hours: the mass of the residue is not more than 0.15%.

(5) Alkali carbonates — To the washings obtained in (4) add 3 drops of methyl orange TS and 2 mL of 0.05 mol/L sulfuric acid VS: a red color develops.

Loss on drying — Not more than 5.0% in the case of the powder, and not more than 10.0% in the case of the granules.

Weigh accurately about 0.5 g of Medicinal Soap in a tared beaker, add 10 g of sea sand (No. 1), previously dried at 105°C for 1 hour, and again weigh the beaker. Add 10 mL of ethanol (95), evaporate on a water bath to dryness with thorough stirring, and dry at 105°C for 3 hours.

Containers and storage — Containers—Well-closed containers.

Mefenamic Acid

Mefenamic Acid occurs as a white to light yellow powder. It is odorless and tasteless at first, but leaves a slightly bitter aftertaste.

\[ C_{15}H_{18}NO_2 \]

2-(2,3-Dimethylphenylamino)benzoic acid

Description Mefenamic Acid occurs as a white to light yellow powder. It is odorless and tasteless at first, but leaves a slightly bitter aftertaste.
It is sparingly soluble in diethyl ether, slightly soluble in methanol, in ethanol (95) and in chloroform, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Melting point: about 225°C (with decomposition).

**Identification (1)** Dissolve 10 mg of Mefenamic Acid in 1 mL of methanol by warming, cool, add 1 mL of a solution of p-nitrobenzene diazonium fluoroborate (1 in 1000) and 1 mL of sodium hydroxide TS, and mix thoroughly: an orange-red color is produced.

(2) Dissolve 10 mg of Mefenamic Acid in 2 mL of sulfuric acid, and heat: the solution shows a yellow color and a green fluorescence.

(3) Dissolve 7 mg of Mefenamic Acid in a solution of hydrochloric acid in methanol (1 in 1000) to make 500 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity (1)** Chloride \(<1.03\>\)—To 1.0 g of Mefenamic Acid add 20 mL of sodium hydroxide TS, and dissolve by warming. Cool, add 2 mL of acetic acid (100) and water to make 100 mL, and mix well. Remove the precipitated material by filtration, discard the first 10 mL of filtrate, and to subsequent 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.01 mol/L hydrochloric acid VS add 5 mL of sodium hydroxide TS, 0.5 mL of acetic acid (100), 6 mL of nitric acid and water to make 50 mL (not more than 0.071%).

(2) Heavy metals \(<1.07\>\)—Proceed with 2.0 g of Mefenamic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic \(<1.11\>\)—Prepare the test solution with 1.0 g of Mefenamic Acid according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Mefenamic Acid, in 5 mL of a mixture of chloroform and methanol (3:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (3:1) to make exactly 200 mL, pipet 10 mL of this solution, add a mixture of chloroform and methanol (3:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.05\>\). Spot 25 \(\mu L\) each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-methyl-1-propanol and ammonia solution (28:3:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying \(<2.42\>\)** Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 4 hours).

**Residue on ignition \(<2.44\>\)** Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Mefenamic Acid, previously dried, and dissolve in 100 mL of ethanol (95), previously neutralized to phenol red TS with 0.1 mol/L sodium hydroxide VS, by warming gently. Cool, and titrate \(<2.50\>\) with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from yellow through yellow-red to red-purple (indicator: 2 to 3 drops of phenol red TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 24.13 mg of C\(_{7}\)H\(_{13}\)NO\(_{2}\).

**Containers and storage** Containers—Well-closed containers.

**Mefloquine Hydrochloride**

メフロキン塩酸塩

C\(_{17}\)H\(_{16}\)F\(_{6}\)N\(_{2}\)O.HCl: 414.77

(1RS)-[2,8-Bis(trifluoromethyl)quinolin-4-yl][2SR]-piperidin-2-yl)methanol monohydrochloride [51773-92-3]

Mefloquine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of C\(_{17}\)H\(_{16}\)F\(_{6}\)N\(_{2}\)O.HCl.

**Description** Mefloquine Hydrochloride occurs as white crystals or a white crystalline powder.

It is freely soluble in methanol, soluble in ethanol (99.5), and slightly soluble in water.

It dissolves in sulfuric acid.

A solution of Mefloquine Hydrochloride in methanol (1 in 20) shows no optical rotation.

Melting point: about 260°C (with decomposition).

**Identification (1)** Dissolve 2 mg of Mefloquine Hydrochloride in 1 mL of sulfuric acid: the solution shows a blue fluorescence under ultraviolet light (main wavelength: 365 nm).

(2) Determine the absorption spectrum of a solution of Mefloquine Hydrochloride in methanol (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Mefloquine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry \(<2.25\>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) To 5 mL of a solution of Mefloquine Hydrochloride (1 in 1000) add 1 mL of dilute nitric acid and 1 mL of silver nitrate TS: a white precipitate is formed, and the separated precipitate dissolves on the addition of an excess amount of...
Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Mefloquine Hydrochloride according to Method 2 using a quartz crucible, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—To 1.0 g of Mefloquine Hydrochloride add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), burn the ethanol, gradually heat, and incinerate by ignition at 800°C. If a carbonized residue still retains, moisten the residue with a little amount of nitric acid, and ignite again to incinerate. After cooling, to the residue add 3 mL of hydrochloric acid, warm on a water bath to dissolve, and perform the test using this solution as the test solution (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Mefloquine Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method; the area of the peak other than mefloquine and the peak eluted first from the sample solution is not larger than 2.5 times the peak area of mefloquine from the standard solution. "System suitability—"

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with aminopropylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of acetonitrile and diluted phosphoric acid (1 in 14) (24:1).

Flow rate: Adjust the flow rate so that the retention time of mefloquine is about 10 minutes.

Time span of measurement: About 3 times as long as the retention time of mefloquine.

System performance: Dissolve 10 mg of mefloquine hydrochloride and 5 mg of diprophylline in 50 mL of the mobile phase. To 2 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, diprophylline and mefloquine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mefloquine is not more than 2.0%.

(4) Residual solvent Using specified separately.

Loss on drying <2.44> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 0.5 g of Mefloquine Hydrochloride, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.3D> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 41.48 mg of C17H16F6N2O.HCl

Containers and storage Containers—Well-closed containers.

Mefruside

**Identification (1)** Determine the absorption spectrum of a solution of Mefruside in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mefruside, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Mefruside as directed under Flame Coloration Test <1.04> (2): a green color appears.

Melting point <2.66> 149 – 152°C
Purity (1) Heavy metals <1.07>—Dissolve 1.0 g of Mefruside in 30 mL of acetone, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 30 mL of acetone, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Mefruside according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.20 g of Mefruside in 10 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>.

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Mefruside, previously dried, dissolve in 80 mL of acetonitrile, 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 \( \mu \)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and acetone (5:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Mefruside, previously dried, dissolve in 80 mL of \( \text{N},\text{N}-\text{dimethylformamide} \) and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Separately, perform a blank determination with a solution prepared by adding 13 mL of water to 80 mL of \( \text{N},\text{N}-\text{dimethylformamide} \), and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS

\[ = 38.29 \text{ mg of C}_{13}\text{H}_{19}\text{ClN}_{2}\text{O}_{5}\text{S}_{2} \]

Containers and storage Containers—Well-closed containers.

Mefruside Tablets
メフルシド錠

Mefruside Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of mefruside (\( \text{C}_{13}\text{H}_{19}\text{ClN}_{2}\text{O}_{5}\text{S}_{2} \) 382.88).

Method of preparation Prepare as directed under Tablets, with Mefruside.

Identification (1) Weigh a quantity of powdered Mefruside Tablets, equivalent to 0.3 g of Mefruside according to the labeled amount, shake with 15 mL of heated methanol for 20 minutes, and filter. Add 25 mL of water to the filtrate, and allow to stand while ice-cooling for 30 minutes. Filter the white precipitate formed, wash with water, and dry at 105°C for 2 hours: the precipitate melts <2.50> between 149°C and 152°C.

(2) Weigh a quantity of powdered Mefruside Tablets, equivalent to 0.01 g of Mefruside according to the labeled amount, shake with 70 mL of methanol for 15 minutes, add methanol to make 100 mL, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>:

- its maxima between 274 nm and 278 nm, and between 283 nm and 287 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Mefruside Tablets add 40 mL of methanol, disintegrate the tablet using ultrasonic waves with occasional stirring, then further treat with ultrasonic waves for 10 minutes, and add methanol to make exactly \( V \) mL of a solution containing about 0.5 mg of mefruside (\( \text{C}_{13}\text{H}_{19}\text{ClN}_{2}\text{O}_{5}\text{S}_{2} \)) per mL. Centrifuge the solution, pipet 5 mL of the supernatant liquid, add methanol to make exactly 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

\[
\text{Amount (mg) of mefruside (C}_{13}\text{H}_{19}\text{ClN}_{2}\text{O}_{5}\text{S}_{2} \}) = M_{S} \times \frac{A_{T}}{A_{S}} \times \frac{20}{V} \times 1/125
\]

\[
M_{S}: \text{Amount (mg) of mefruside for assay}
\]

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Mefruside Tablets is not less than 85%.

Start the test with 1 tablet of Mefruside Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a filter paper for quantitative analysis (SC). Discard the first 5 mL of the filtrate, pipet \( V \) mL of the subsequent filtrate, add water to make exactly \( V \) mL so that each mL contains about 28 \( \mu \)g of mefruside (\( \text{C}_{13}\text{H}_{19}\text{ClN}_{2}\text{O}_{5}\text{S}_{2} \)) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 70 mg of mefruside for assay, previously dried at 105°C for 2 hours, dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_{S} \) and \( A_{T} \), of the sample solution and standard solution at 285 nm in a layer of 5 cm in length as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

Dissolution rate (%) with respect to the labeled amount of mefruside (\( \text{C}_{13}\text{H}_{19}\text{ClN}_{2}\text{O}_{5}\text{S}_{2} \))

\[
= M_{S} \times \frac{A_{T}}{A_{S}} \times V \times V \times 1/C \times 36
\]

\[
M_{S}: \text{Amount (mg) of mefruside for assay}
\]

C: Labeled amount (mg) of mefruside (\( \text{C}_{13}\text{H}_{19}\text{ClN}_{2}\text{O}_{5}\text{S}_{2} \)) in 1 tablet

Assay Weigh accurately not less than 20 Mefruside Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 65 mg of mefruside (\( \text{C}_{13}\text{H}_{19}\text{ClN}_{2}\text{O}_{5}\text{S}_{2} \)), shake with 70 mL of methanol for 15 minutes, then add methanol to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, take exactly 10 mL of the subsequent filtrate, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 65 mg of mefruside for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution,
add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, \( A_T \) and \( A_S \), of the sample solution and standard solution at 285 nm as directed under Ultraviolet-visible Spectrophotometry (2.29).

\[
\text{Amount (mg) of mefruside (C}_{13}\text{H}_{19}\text{ClN}_2\text{O}_5\text{S}_2) = M_S \times A_T / A_S
\]

\( M_S \): Amount (mg) of mefruside for assay

Containers and storage  Containers—Tight containers.

**Meglumine**  メグルミン

![Meglumine structure](image)

\( \text{C}_{17}\text{H}_{21}\text{NO}_5: 195.21 \)

1-Deoxy-1-methylamino-D-glucitol  

[6284-40-8]

Meglumine, when dried, contains not less than 99.0% of \( \text{C}_{17}\text{H}_{21}\text{NO}_5 \).

Description  メグルミン occurs as a white, crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in water, and slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Meglumine (1 in 10) is between 11.0 and 12.0.

Identification (1)  To 1 mL of a solution of Meglumine (1 in 10) add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS: a deep red color develops.

(2)  To 2 mL of a solution of Meglumine (1 in 10) add 1 drop of methyl red TS, and add 0.5 mL of dilute sodium hydroxide TS and 0.5 g of boracic acid after neutralizing with 0.5 mol/L sulfuric acid TS: a deep red color develops.

(3)  Dissolve 0.5 g of Meglumine in 1 mL of dilute hydrochloric acid (1 in 3), and add 10 mL of ethanol (99.5): a white precipitate is produced. Then, rubbing the inside wall of the container with a glass rod, cool with ice and produce more precipitate. Filter the precipitate by suction through a glass filter (G3), wash the precipitate with a small volume of ethanol (99.5), and dry at 105°C for 1 hour: the residue thus obtained melts \(<2.60^\circ\) between 149°C and 152°C.

Optical rotation (2.29)  \([\alpha]_D^20\) = -16.0 to -17.0° (after drying, 1 g, water, 10 mL, 100 mm)

Melting point (2.26)  128 – 131°C

Purity (1)  Clarity and color of solution—Dissolve 1.0 g of Meglumine in 10 mL of water: the solution is clear and colorless.

(2)  Chloride (1.03)—Dissolve 1.0 g of Meglumine in 30 mL of water, and add 10 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.009%).

(3)  Sulfate (1.14)—Dissolve 1.0 g of Meglumine in 30 mL of water, and add 5 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

(4)  Heavy metals (1.07)—Proceed with 2.0 g of Meglumine according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5)  Arsenic (1.11)—Prepare the test solution with 2.0 g of Meglumine according to Method 3, and perform the test (not more than 1 ppm).

(6)  Reducing substances—To 5 mL of a solution of Meglumine (1 in 20) add 5 mL of Fehling’s TS, and boil for 2 minutes: no red-brown precipitate is produced.

Loss on drying (2.41)  Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition (2.44)  Not more than 0.1% (1 g).

Assay  Weigh accurately about 0.4 g of Meglumine, previously dried, dissolve in 25 mL of water, and titrate with 0.1 mol/L hydrochloric acid VS (indicator: 2 drops of methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS = 19.52 mg of \( \text{C}_{17}\text{H}_{21}\text{NO}_5 \)

Containers and storage  Containers—Tight containers.

**Meglumine Iotalamate Injection**  イオタラム酸メグルミン注射液

Meglumine Iotalamate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of iotalamic acid (\( \text{C}_{11}\text{H}_{9}\text{I}_3\text{N}_2\text{O}_4\): 613.91).

Method of preparation

(1)  Iotalamic Acid  227.59 g

Meglumine  72.41 g

Water for Injection or Sterile Water  for Injection in Containers  a sufficient quantity

To make 1000 mL

(2)  Iotalamic Acid  455 g

Meglumine  145 g

Water for Injection or Sterile Water  for Injection in Containers  a sufficient quantity

To make 1000 mL

Prepare as directed under Injections, with the above ingredients (1) or (2).

Description  Meglumine Iotalamate Injection is a clear, colorless to pale yellow, slightly viscous liquid.

It gradually changes in color by light.

Identification (1)  To 1 mL of Meglumine Iotalamate Injection add 1 mL of potassium naphthoquinone sulphonate TS and 0.2 mL of sodium hydroxide TS: a deep red color develops.
(2) To a volume of Meglumine Iotalamate Injection, equivalent to 1 g of Iotalamic Acid according to the labeled amount, add 25 mL of water, and add 2.5 mL of dilute hydrochloric acid while shaking: a white precipitate is produced. Filter the precipitate by suction through a glass filter (G4), wash the precipitate with two 10-mL portions of water, and dry at 105°C for 4 hours. Proceed with the precipitate so obtained as directed in the Identification (2) under Iotalamate Acid.

**Optical rotation** \(<2.49\)

Method of preparation (1) \(\alpha_D^{20} = -1.67 - -1.93^\circ\) (100 mm).

Method of preparation (2) \(\alpha_D^{20} = -3.35 - -3.86^\circ\) (100 mm).

**pH** \(<2.54\> 6.5 - 7.7

**Purity (1)** Primary aromatic amines—To a volume of Meglumine Iotalamate Injection, equivalent to 0.20 g of Iotalamic Acid according to the labeled amount, add 15 mL of water, shake, add 4 mL of a solution of sodium nitrite (1 in 100) under ice-cooling, and proceed as directed in the Purity (2) under Iotalamate Acid: the absorbance is not more than 0.17.

(2) Iodine and iodide—Take a volume of Meglumine Iotalamate Injection, equivalent to 1.5 g of Iotalamic Acid according to the labeled amount, and proceed as directed in the Purity (2) under Sodium Iotalamate Injection.

**Extractable volume** \(<6.05\> It meets the requirement.

**Foreign insoluble matter** \(<6.06\> Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** \(<6.07\> It meets the requirement.

**Sterility** \(<4.06\> Perform the test according to the Membrane filtration method: it meets the requirements.

**Assay** To an exactly measured volume of Meglumine Iotalamate Injection, equivalent to about 4 g of Iotalamic acid (C\(_{11}\)H\(_9\)I\(_3\)N\(_2\)O\(_4\)), add water to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 200 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of Iotalamic acid for assay, previously dried at 105°C for 4 hours, dissolve in 100 mL of water and 1 mL of sodium hydroxide TS, and add water to make exactly 200 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 10 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\> according to the following conditions, and calculate the ratios, \(Q_I\) and \(Q_S\), of the peak area of Iotalamic acid to that of the internal standard.

\[
\text{Amount (mg) of Iotalamic acid (C}_{11}\text{H}_{9}\text{I}_{3}\text{N}_{2}\text{O}_{4}) = M_S \times Q_I / Q_S \times 10
\]

\(M_S\): Amount (mg) of Iotalamic acid for assay

**Internal standard solution**—A solution of L-tryptophan in the mobile phase (3 in 2500).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: Dissolve 3.9 g of phosphoric acid and 2.8 mL of triethylamine in water to make 2000 mL. To this solution add 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of Iotalamic acid is about 6 minutes.

**System suitability**—

System performance: When the procedure is run with 10 \(\mu\)L of the standard solution under the above operating conditions, Iotalamic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of Iotalamic acid to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

**Meglumine Sodium Amidotrizoate Injection**

アミドトリゾ酸ナトリウムメグルミン注射液

Meglumine Sodium Amidotrizoate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of amidotrizoic acid (C\(_{11}\)H\(_9\)I\(_3\)N\(_2\)O\(_4\)): 613.91).

**Method of preparation**

(1)

| Amidotrizoic Acid (anhydrous) | 471.78 g |
| Sodium Hydroxide | 5.03 g |
| Meglumine | 125.46 g |

**Water for Injection or Sterile Water**

| for Injection in Containers | a sufficient quantity |

To make 1000 mL

(2)

| Amidotrizoic Acid (anhydrous) | 597.30 g |
| Sodium Hydroxide | 6.29 g |
| Meglumine | 159.24 g |

**Water for Injection or Sterile Water**

| for Injection in Containers | a sufficient quantity |

To make 1000 mL

Prepare as directed under Injections, with the above ingredients (1) or (2).

**Description** Meglumine Sodium Amidotrizoate Injection is a clear, colorless to pale yellow, slightly viscous liquid.
It gradually changes in color by light.

Identification (1) To a volume of Meglumine Sodium Amidotrizoate Injection, equivalent to 1 g of Amidotrizoic Acid according to the labeled amount, add 25 mL of water, and add 2.5 mL of dilute hydrochloric acid with stirring: a white precipitate is produced. Filter the precipitate by suction through a glass filter (G4), wash with two 10-mL portions of water, and dry at 105°C for 1 hour. Proceed with the precipitate so obtained as directed in the Identification (2) under Amidotrizoic Acid.

(2) To 1 mL of Meglumine Sodium Amidotrizoate Injection add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color develops in the chloroform layer. Then add 1 mL of chloroform to the filtrate, and shake vigorously: no color develops in the chloroform layer. Then add 1 mL of potassium iodide in water to make exactly 100 mL, then pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use this solution as the sample solution. Separately, perform the test with 5 μL of each of the sample solution and standard solution as directed under Liquid Chromatography (2.012) according to the following conditions, and calculate the ratios, Q₁ and Q₀, of the peak area of amidotrizoic acid to that of the internal standard.

Amount (mg) of amidotrizoic acid (C₁₁H₉I₃N₂O₄) = \( M_S \times \frac{Q_1}{Q_0} \times 2 \)

\( M_S \): Amount (mg) of amidotrizoic acid for assay, calculated on the dried basis

Internal standard solution—Dissolve 0.06 g of acetrizoic acid in a solution of meglumine (3 in 1000) to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.7 g of tetrabutylammonium phosphate and 7.0 g of dipotassium hydrogenphosphate in 750 mL of water, adjust the pH to 7.0 with diluted phosphoric acid (1 in 10), add water to make 800 mL, then add 210 mL of acetonitrile, and mix.

Flow rate: Adjust the flow rate so that the retention time of amidotrizoic acid is about 5 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, amidotrizoic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of amidotrizoic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Meglumine Sodium Iodamide Injection

ヨーダミドノトリウムメグルミン注射液

Meglumine Sodium Iodamide Injection is an aqueous solution for injection.

It contains not less than 59.7 w/v% and not more than 65.9 w/v% of iodamide (C₁₂H₁₁I₃N₂O₄: 627.94).
Method of preparation

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodamide</td>
<td>627.9 g</td>
</tr>
<tr>
<td>Sodium Hydroxide</td>
<td>6.0 g</td>
</tr>
<tr>
<td>Meglumine</td>
<td>165.9 g</td>
</tr>
<tr>
<td>Water for Injection or Sterile Water</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

Description Meglumine Sodium Iodamide Injection is a clear, colorless to pale yellow, slightly viscous liquid. It gradually changes in color by light.

Identification (1) To 2 mL of Meglumine Sodium Iodamide Injection add 25 mL of water, and add 3 mL of dilute hydrochloric acid with thorough stirring: a white precipitate is formed. Filter the precipitate by suction through a glass filter (G3), and wash with two 10-mL portions of water. Transfer the precipitate to a suitable flask, add 100 mL of water, dissolve by heating, and gently boil until the volume becomes about 30 mL. After cooling, collect the separated crystals by filtration, dry at 105°C for 1 hour, and proceed as directed in the Identification (1) and (2) under Iodamide.

(2) Determine the infrared absorption spectrum of the dried crystals obtained in (1) as directed in the potassium bromide disk method under Infrared Spectrophotometry <2,25>: it exhibits absorption at the wave numbers of about 3390 cm⁻¹, 1369 cm⁻¹, 1296 cm⁻¹, 1210 cm⁻¹ and 1194 cm⁻¹.

(3) To 1 mL of Meglumine Sodium Iodamide Injection add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color is produced.

(4) Meglumine Sodium Iodamide Injection responds to the Qualitative Tests <1,19> (1) for sodium salt.

Optical rotation <2,49> αD: −3.84 ± 4.42° (100 mm).

pH <2,54> 6.5 – 7.5

Purity (1) Primary aromatic amines—Mix 0.30 mL of Meglumine Sodium Iodamide Injection and 6 mL of water, then add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, shake well, and proceed as directed in the Purity (2) under Iodamide: the absorbance is not more than 0.22.

(2) Iodine and iodide—To 0.40 mL of Meglumine Sodium Iodamide Injection add water to make 20 mL, then add 5 mL of dilute nitric acid, shake well, filter by suction through a glass filter (G3). To the filtrate add 5 mL of chloroform, and shake vigorously: no color develops in the chloroform layer. Then add 1 mL of a strong hydrogen peroxide solution, and shake vigorously: the chloroform layer has no more color than the control solution.

Control solution: Dissolve 0.10 g of potassium iodide in water to make 100 mL. To a 0.10-mL portion of this solution add 20 mL of water, 5 mL of dilute nitric acid, 5 mL of chloroform and 1 mL of strong hydrogen peroxide solution, and shake vigorously.

Extractable volume <6.05> It meets the requirement.

Pyrogen <4.04> Dilute Meglumine Sodium Iodamide Injection with isotonic sodium chloride solution so as to contain 0.30 mL of Meglumine Sodium Iodamide Injection per mL according to the labeled amount, and perform the test: it meets the requirements.

Assay To an exactly measured 8 mL of Meglumine Sodium Iodamide Injection add sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample solution. Pipet 10 mL of the sample solution into a saponification flask, add 30 mL of sodium hydroxide TS and 1 g of zinc powder, and proceed as directed in the Assay under Iodamide.

Each mL of 0.1 mol/L silver nitrate VS = 20.93 mg of C13H11I3N2O4

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Melphalan

メルファラン

C13H18Cl2N2O2: 305.20
4-Bis(2-chloroethyl)amino-L-phenylalanine [148-82-3]

Melphalan contains not less than 93.0% of C13H18Cl2N2O2, calculated on the dried basis.

Description Melphalan occurs as a white, to light yellowish white, crystalline powder. It is slightly soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether. It dissolves in dilute hydrochloric acid and in dilute sodium hydroxide TS. It is gradually colored by light.

Optical rotation [α]D: about −32° (0.5 g, calculated on the dried basis, methanol, 100 mL, 100 mm).

Identification (1) To 20 mg of Melphalan add 50 mL of methanol, dissolve by warming, add 1 mL of a solution of 4-(4-nitrobenzyl)pyridine in acetone (1 in 20), and evaporate on a water bath to dryness. Dissolve the residue in 1 mL of warmed methanol and add 2 drops of ammonia solution (28): a purple color develops.

(2) Dissolve 0.1 g of Melphalan in 10 mL of dilute sodium hydroxide TS, and heat on a water bath for 10 minutes. After cooling, add dilute nitric acid to acidify, and filter: the filtrate responds to the Qualitative Tests <1,19> for chloride.

(3) Determine the absorption spectrum of a solution of Melphalan in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2,24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Ionisable chloride—Weigh accurately about 0.5 g of Melphalan, dissolve in 80 mL of dilute nitric acid
(1 in 40), stir for 2 minutes, and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration): the consumed volume is not more than 1.0 mL to 0.50 g of Melphalan.

(2) Heavy metals \(<1.07\>—Proceed with 1.0 g of Melphalan according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic \(<1.11\>— Prepare the test solution with 1.0 g of Melphalan according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying \(<2.41\>\ Not more than 7.0% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, \(105^\circ\)C, 2 hours).

Residue on ignition \(<2.44\>\ Not more than 0.3% (1 g).

Assay Weigh accurately about 0.25 g of Melphalan, add 20 mL of a solution of potassium hydroxide (1 in 5), and heat under a reflux condenser on a water bath for 2 hours. After cooling, add 75 mL of water and 5 mL of nitric acid, cool, and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration). Make any necessary correction by using the results obtained in the Purity (1).

Each mL of 0.1 mol/L silver nitrate VS = 15.26 mg of \(\text{C}_{13}\text{H}_{18}\text{Cl}_{2}\text{N}_{2}\text{O}_{2}\)

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Menatetrenone

メナテトレノン

\[
\text{C}_{31}\text{H}_{40}\text{O}_{2}\; (444,65)
\]

2-Methyl-3-[(2E,6E,10E)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-yl]-1,4-naphthoquinone

[863-61-6]

Menatetrenone contains not less than 98.0% of \(\text{C}_{31}\text{H}_{40}\text{O}_{2}\), calculated on the dehydrated basis.

Description Menatetrenone occurs as yellow, crystals, crystalline powder, waxy mass or oily material.

It is very soluble in hexane, soluble in ethanol (99.5), sparingly soluble in 2-propanol, slightly soluble in methanol, and practically insoluble in water.

It decomposes and the color becomes more intense by light.

Melting point: about \(37^\circ\)C.

Identification (1) Dissolve 0.1 g of Menatetrenone in 5 mL of ethanol (99.5) by warming, cool, and add 1 mL of a solution of potassium hydroxide in ethanol (95) (1 in 10): a blue color develops, and upon standing it changes from blue-purple to red-brown through red-purple.

(2) Determine the infrared absorption spectrum of Menatetrenone, after melting by warming if necessary, as directed in the liquid film method under Infrared Spectrophotometry \(<2.25\>\, and compare the spectrum with the Reference Spectrum or the spectrum of Menatetrenone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals \(<1.07\>—Proceed with 1.0 g of Menatetrenone according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Menadione—To 0.20 g of Menatetrenone add 5 mL of diluted ethanol (1 in 2), shake well, and filter. To 0.5 mL of the filtrate add 1 drop of a solution of 3-methyl-1-phenyl-5-pyrazorone in ethanol (99.5) (1 in 20) and 1 drop of ammonia water (28), and allow to stand for 2 hours: no blue-purple color develops.

(3) cis Isomer—Dissolve 0.10 g of Menatetrenone in 10 mL of hexane, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add hexane to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.05\>\, Spot 10 \mu L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the chromatogram with a mixture of hexane and dibutyl ether (17:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot corresponding to relative RF value 1.1 regarding to the principal spot from the sample solution is not more intense than the spot from the standard solution.

(4) Related substances—Conduct this procedure without exposure to daylight, using a light-resistant vessel. Dissolve 0.10 g of Menatetrenone in 100 mL of ethanol (99.5), and use this solution as the standard solution. Pipet 1 mL of the sample solution, add ethanol (99.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 \(\mu L\) each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.06\>\, according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of peaks other than the peak of menatetrenone from the sample solution is not larger than the peak area of menatetrenone from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 6 times as long as the retention time of menatetrenone beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add ethanol (99.5) to make exactly 50 mL. Confirm that the peak area of menatetrenone obtained from 20 \(\mu L\) of this solution is equivalent to 7 to 13% of that from 20 \(\mu L\) of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 \(\mu L\) of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of menatetrenone is not more than 1.0%.

Water \(<2.48\>\ Not more than 0.5% (0.5 g, volumetric titration, direct titration).
Residue on ignition 2.44 Not more than 0.1% (1 g).

Assay Conduct this procedure without exposure to daylight, using a light-resistant vessel. Weigh accurately about 0.1 g each of Menatetrenone and Menatetrenone RS (separately, determine the water 2.46 in the same manner as Menatetrenone), dissolve each in 50 mL of 2-propanol, and add ethanol (99.5) to make exactly 100 mL. Pipet 10 mL of these solutions, and add ethanol (99.5) to make exactly 100 mL. Pipet 2 mL each of these solutions, add exactly 4 mL of 2-propanol (1 in 20,000), and use these solutions as the sample solution and standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the ratios, Q1 and Q8, of the peak area of menatetrenone to that of the internal standard.

Amount (mg) of C9H18O = M8 × Q1/Q8

M8: Amount (mg) of Menatetrenone RS, calculated on the dehydrated basis

Internal standard solution—A solution of phytonadione in 2-propanol (1 in 20,000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 270 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Methanol.
Flow rate: Adjust the flow rate so that the retention time of menatetrenone is about 7 minutes.
System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, menatetrenone and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of menatetrenone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

dl-Menthol
dl-メントール

C10H20O: 156.27
(1RS,2SR,5RS)-5-Methyl-2-(1-methylethyl)cyclohexanol [89-78-1]
dl-Menthol contains not less than 98.0% of C10H20O.

Description dl-Menthol occurs as colorless crystals. It has a characteristic and refreshing odor and a burning taste, followed by a cool taste.

It is very soluble in ethanol (95) and in diethyl ether, and very slightly soluble in water.

It sublimes gradually at room temperature.

Identification (1) Triturate dl-Menthol with an equal amount of camphor, chloral hydrate or thymol: the mixture liquefies.

(2) Shake 1 g of dl-Menthol with 20 mL of sulfuric acid: the mixture becomes turbid with a yellow-red color. Allow to stand for 3 hours: a clear, oily layer possesses no aroma of menthol is separated.

Congealing point 2.42 27–28°C

Optical rotation 2.49 [α]D25: −2.0 – +2.0° (2.5 g, ethanol (95), 25 mL, 100 mm).

Purity (1) Non-volatile residue—Volatilize 2.0 g of dl-Menthol on a water bath, and dry the residue at 105°C for 2 hours: the residue weighs not more than 1.0 mg.

(2) Thymol—Add 0.20 g of dl-Menthol to a cold mixture of 2 mL of acetic acid (100), 6 drops of sulfuric acid and 2 drops of nitric acid: no green to blue-green color immediately develops.

(3) Nitromethane or nitroethane—To 0.5 g of dl-Menthol placed in a flask add 2 mL of a solution of sodium hydroxide (1 in 2) and 1 mL of strong hydrogen peroxide, connect a reflux condenser to the flask, and boil the mixture gently for 10 minutes. After cooling, add water to make exactly 20 mL, and filter. Take 1 mL of the filtrate in a Nessler tube, add water to make 10 mL, neutralize with dilute hydrochloric acid, then add 1 mL of dilute hydrochloric acid, and cool. To the mixture add 1 mL of a solution of sulfanilic acid (1 in 100), allow to stand for 2 minutes, and then add 1 mL of a solution of N,N-diethyl-NN'-1-naphthylethylenediamine oxalate (1 in 1000) and water to make 25 mL: no red-purple color immediately develops.

Assay Weigh accurately about 2 g of dl-Menthol, add exactly 20 mL of a mixture of dehydrated pyridine and acetic anhydride (8:1), connect a reflux condenser, and heat on a water bath for 2 hours. Wash down the condenser with 20 mL of water, and titrate 2.50D with 1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.
Each mL of 1 mol/L sodium hydroxide VS
= 156.3 mg of C_{10}H_{20}O

Containers and storage Containers—Tight containers.
Storage—In a cold place.

l-Menthol
l-メントール

C_{10}H_{20}O: 156.27
(1R,2S,5R)-5-Methyl-2-(1-methylethyl)cyclohexanol
[2216-51-5]

l-Menthol contains not less than 98.0% of C_{10}H_{20}O.

Description l-Menthol occurs as colorless crystals. It has a characteristic and refreshing odor and a burning taste, followed by a cool taste.

It is very soluble in ethanol (95) and in diethyl ether, and very slightly soluble in water.

It sublimes gradually at room temperature.

Identification (1) Triturate l-Menthol with an equal amount of camphor, chloral hydrate or thymol: the mixture liquefies.

(2) Shake 1 g of l-Menthol with 20 mL of sulfuric acid: the mixture becomes turbid with a yellow-red color. Allow to stand for 3 hours: a clear, oily layer which possesses no aroma of menthol is separated.

Optical rotation <2.49 > [α]_{D}^{20} = -45.0 to -51.0° (2.5 g, ethanol (95), 25 mL, 100 mm).

Melting point <2.60> 42 – 44°C

Purity (1) Non-volatile residue—Volatilize 2.0 g of l-Menthol on a water bath, and dry the residue at 105°C for 2 hours: the residue weighs not more than 1.0 mg.

(2) Thymol—Add 0.20 g of l-Menthol to a cold mixture of 2 mL of acetic acid (100), 6 drops of sulfuric acid and 2 drops of nitric acid: no green to blue-green color immediately develops.

(3) Nitromethane or nitroethane—To 0.5 g of l-Menthol placed in a flask add 2 mL of sodium hydroxide solution (1 in 2) and 1 mL of strong hydrogen peroxide, connect a reflux condenser to the flask, and boil the mixture gently for 10 minutes. After cooling, add water to make exactly 20 mL, and filter. Take 1 mL of the filtrate in a Nessler tube, add water to make 10 mL, neutralize with dilute hydrochloric acid, add another 1 mL of dilute hydrochloric acid, and cool. To the mixture add 1 mL of a solution of sulfanilic acid (1 in 100), allow to stand for 2 minutes, and then add 1 mL of a solution of N,N-diethyl-N'-1-naphthylethlenediamine oxalate (1 in 1000) and water to make 25 mL: no red-purple color immediately develops.

Assay Weigh accurately about 2 g of l-Menthol, add exactly 20 mL of a mixture of dehydrated pyridine and acetic anhydride (8:1), connect a reflux condenser, and heat on a water bath for 2 hours. Wash the condenser with 20 mL of water, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS
= 156.3 mg of C_{10}H_{20}O

Containers and storage Containers—Tight containers.
Storage—In a cold place.

Mepenzolate Bromide
メペンゾラート臭化物

C_{21}H_{26}BrNO_3: 420.34
(3RS)-3-[[[(Hydroxy)(diphenyl)acetoxyl]-1,1-dimethylpiperidinium bromide
[76-90-4]

Mepenzolate Bromide, when dried, contains not less than 98.5% of C_{21}H_{26}BrNO_3.

Description Mepenzolate Bromide is white to pale yellow crystals or crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in formic acid, freely soluble in methanol, soluble in hot water, slightly soluble in water and in ethanol (95), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Melting point: about 230°C (with decomposition).

Identification (1) To 30 mg of Mepenzolate Bromide add 10 drops of sulfuric acid: a red color develops.

(2) Dissolve 10 mg of Mepenzolate Bromide in 20 mL of water and 5 mL of dilute hydrochloric acid, and to 5 mL of this solution add 1 mL of Dragendorff’s TS: an orange precipitate is produced.

(3) Nitromethane or nitroethane—To 0.5 g of Mepenzolate Bromide placed in a flask add 2 mL of sodium hydroxide solution (1 in 2) and 1 mL of strong hydrogen peroxide, connect a reflux condenser to the flask, and boil the mixture gently for 10 minutes. After cooling, add water to make exactly 20 mL, and filter. Take 1 mL of the filtrate in a Nessler tube, add water to make 10 mL, neutralize with dilute hydrochloric acid, add another 1 mL of dilute hydrochloric acid, and cool. To the mixture add 1 mL of a solution of sulfanilic acid (1 in 100), allow to stand for 2 minutes, and then add 1 mL of a solution of N,N-diethyl-N'-1-naphthylethlenediamine oxalate (1 in 1000) and water to make 25 mL: no red-purple color immediately develops.

(4) Dissolve 0.5 g of Mepenzolate Bromide in 50 mL of water and 3 mL of nitric acid by heating. This solution responds to the Qualitative Tests <1.09> for Bromide.

Purity (1) Heavy Metals <1.07>—Proceed with 1.0 g of Mepenzolate Bromide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not less than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Mepenzolate Bromide according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.40 g of Mepenzolate Bromide in exactly measured 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use
this solution as the standard solution (1). Separately, dissolve 40 mg of benzophenone in methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 10 µL each of the sample solution, standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, methanol, water and acetic acid (100:3:3:2:1) to a distance of about 10 cm, and air-dry the plate and then at 80°C for 30 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than either the principal spot or the spot corresponding to benzophenone from the sample solution are not more intense than the spot from standard solution (1), and the spot corresponding to benzophenone from the sample solution is not more intense than the spot from standard solution (2). Spray evenly Dragendorff’s TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from standard solution (1).

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.35 g of Mepenzolate Bromide, previously dried, dissolve in 2 mL of formic acid, and titrate Bromide, previously dried, dissolve in 2 mL of formic acid,

**Containers and storage** Containers—Tight containers.

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**Mepitiostane**

メピチオスタン

C_{25}H_{40}O_{2}S: 404.65

2α,3α-Epithio-17β-(1-methoxycyclopentyloxy)-5α-androstane

[M1326-69-6] (2)

Mepitiostane contains not less than 96.0% and not more than 102.0% of C_{25}H_{40}O_{2}S, calculated on the anhydrous basis.

**Description** Mepitiostane occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in triethylamine, in chloroform, in diethyl ether and in cyclohexane, soluble in diethylene glycol dimethyl ether and in petroleum ether, sparingly soluble in acetone, slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It is hydrolyzed in moist air.

**Identification** (1) Dissolve 1 mg of Mepitiostane in 1 mL of methanol, and add 0.5 mL of palladium (II) chloride TS: an orange precipitate is formed. To this suspension add 1 mL of water and 2 mL of chloroform, shake well, and allow to stand: an orange color develops in the chloroform layer.

(2) Dissolve 0.1 g of Mepitiostane in 2 mL of diethylene glycol dimethyl ether, shake with 1 mL of 1 mol/L hydrochloric acid TS, and filter. To the filtrate add 1.5 mL of 2,4-dinitrophenylhydrazine-diethylene glycol dimethyl ether TS and 1.5 mL of diluted ethanol (95) (2 in 3): an orange-yellow precipitate is formed. Filter the precipitate, recrystallize from ethanol (99.5), and dry in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours: the crystals melt 2<.60> between 144°C and 149°C.

(3) Determine the infrared absorption spectrum of Mepitiostane as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> [α]_{D}^{20} +20 – +23° (0.1 g, chloroform, 10 mL, 100 mm).

**Purity** (1) Clarity and color of solution—Dissolve 0.10 g of Mepitiostane in 4 mL of petroleum ether: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Mepitiostane according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 20 mg of Mepitiostane in exactly 5 mL of a mixture of acetone and triethylamine (1000:1), and use this solution as the sample solution. Separately, dissolve 10 mg of Epitiostanol RS in a mixture of acetone and triethylamine (1000:1) to make exactly 10 mL. Pipet 1 mL and 3 mL of this solution, to each add a mixture of acetone and triethylamine (1000:1) to make exactly 25 mL, and use these solutions as the standard solution (1) and the standard solution (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 5 µL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 5) on the plate, heat between 120°C and 130°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the sample solution showing the same Rf value as the standard solutions are not more intense than the spot from the standard solution (2), and the remaining spots other than the principal spot are not more intense than the spot from the standard solution (1).

**Water** <2.48> Not more than 0.7% (0.3 g, volumetric titration, back titration).

**Residue on ignition** <2.44> Not more than 0.1% (0.5 g).

**Assay** Weigh accurately about 0.3 g of Mepitiostane, and dissolve in cyclohexane to make exactly 10 mL. Pipet 2 mL
of this solution, add 10 mL of ethanol (99.5), mix with exactly 2 mL each of 0.01 mol/L hydrochloric acid TS and the internal standard solution, add ethanol (99.5) to make 20 mL, allow to stand at ordinary temperature for 30 minutes, and use this solution as the sample solution. Separately, weigh accurately about 45 mg of Epitiostanol RS, dissolve in exactly 2 mL of the internal standard solution, add ethanol (99.5) to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_T \) and \( Q_S \), of the peak area of epitiostanol to that of the internal standard, respectively.

\[
\text{Amount (mg) of C}_{25}\text{H}_{40}\text{O}_{2}\text{S} = M_S \times \frac{Q_T}{Q_S} \times 5 \times 1.320
\]

\( M_S \): Amount (mg) of Epitiostanol RS, calculated on the anhydrous basis

**Internal standard solution**—A solution of n-octylbenzene in ethanol (99.5) (1 in 300).

**Operating conditions**—
- Detector: An ultraviolet absorption photometer (wavelength: 265 nm).
- Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).
- Column temperature: A constant temperature of about 25°C.
- Mobile phase: A mixture of methanol and water (20:3).
- Flow rate: Adjust the flow rate so that the retention time of epitiostanol is about 6 minutes.

**System suitability**—
- System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, epitiostanol and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.
- System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of epitiostanol to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Hermetic containers.
- Storage—Light-resistant, under Nitrogen atmosphere, and in a cold place.

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**Mepivacaine Hydrochloride**

メピバカイン塩酸塩

\[
\text{C}_{15}\text{H}_{22}\text{N}_{2}\text{O}.\text{HCl}: 282.81
\]

\( \text{C}_{15}\text{H}_{22}\text{N}_{2}\text{O}.\text{HCl}. \)

Mepivacaine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of \( \text{C}_{15}\text{H}_{22}\text{N}_{2}\text{O}.\text{HCl}. \)

**Description**—Mepivacaine Hydrochloride occurs as white crystals or crystalline powder.
- It is freely soluble in water and in methanol, soluble in acetic acid (100), and sparingly soluble in ethanol (99.5).
- A solution of Mepivacaine Hydrochloride (1 in 10) shows no optical rotation.
- Melting point: about 256°C (with decomposition).

**Identification (1)**—Determine the absorption spectrum of a solution of Mepivacaine Hydrochloride (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mepivacaine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Mepivacaine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

**pH** <2.54>—Dissolve 0.2 g of Mepivacaine Hydrochloride in 10 mL of water: the pH of this solution is between 4.0 and 5.0.

**Purity (1)**—Clarity and color of solution—Dissolve 1.0 g of Mepivacaine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 0.5 g of Mepivacaine Hydrochloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Mepivacaine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 0.10 g of Mepivacaine Hydrochloride in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 2 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard
Weigh accurately about 0.4 g of Mepivacaine Hydrochloride, dried at 105°C for 3 hours.

Assay

Weigh accurately about 0.4 g of Mepivacaine Hydrochloride as directed under Injections, equivalent to about 40 mg of mepivacaine hydrochloride (C15H22N2O.HCl), add exactly 4 mL of the internal standard solution and 0.001 mol/L hydrochloric acid TS to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of mepivacaine hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in 0.001 mol/L hydrochloric acid TS, add exactly 4 mL of the internal standard solution and 0.001 mol/L hydrochloric acid TS to make 20 mL, and use this solution as the standard solution. Perform the test with 5 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q7 and Q8, of the peak area of mepivacaine to that of the internal standard.

\[
M_S: \text{Amount (mg) of mepivacaine hydrochloride (C}_{15}\text{H}_{22}\text{N}_2\text{O}.\text{HCl}) = \frac{M_S}{Q_T/Q_S}
\]

Internal standard solution—A solution of benzophenone in methanol (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.88 g of sodium lauryl sulfate in 1000 mL of a mixture of 0.02 mol/L phosphate buffer solution, pH 3.0, and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of mepivacaine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 5 µL of the standard solution under the above operating conditions, mepivacaine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 5 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of mepivacaine to that of the internal standard is not more than 1.0%.

Containers and storage

Containers—Tight containers.
Mequitazine  

メキタジン

[C_{20}H_{22}N_{2}S: 322.47  
10-[(3R,5)-1-Azabicyclo[2.2.2]oct-3-ylmethyl]-10H- 
phenothiazine]  
[29216-28-2]

Mequitazine, when dried, contains not less than 98.5% of C_{20}H_{22}N_{2}S.

**Description**  
Mequitazine occurs as white crystals or crystalline powder.

It is freely soluble in methanol and in acetic acid (100), soluble in ethanol (95), and practically insoluble in water.

It is gradually colored by light.

A solution of Mequitazine in methanol (1 in 50) shows no optical rotation.

**Identification** (1)  
Determine the absorption spectrum of a solution of Mequitazine in ethanol (95) (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mequitazine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <2.60> 146 - 150°C

**Purity** (1)  
Heavy metals <1.07>—Proceed with 1.0 g of Mequitazine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 50 mg of Mequitazine in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, then pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop with a mixture of ethyl acetate, methanol and diethylamine (7:2:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spot other than the principal spot from the sample solution is not more than 3 and they are not more intense than the spot from the standard solution.

**Loss on drying** <2.4> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

**Residue on ignition** <2.4> Not more than 0.1% (1 g).

**Assay**  
Weigh accurately about 0.25 g of Mequitazine, dissolve in 50 mL of acetic acid (100), titrate <2.59> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 32.25 mg of C_{20}H_{22}N_{2}S

**Containers and storage**  
Containers—Tight containers. Storage—Light-resistant.

**Mercaptopurine Hydrate**  

メルカプトプリン水和物

C_{8}H_{7}N_{3}S.H_{2}O: 170.19  
1,7-Dihydro-6H-purine-6-thione monohydrate  
[6112-76-1]

Mercaptopurine Hydrate contains not less than 98.0% of mercaptopurine (C_{5}H_{4}N_{4}S: 152.18), calculated on the anhydrous basis.

**Description**  
Mercaptopurine Hydrate occurs as light yellow to yellow crystals or crystalline powder. It is odorless.

It is practically insoluble in water, in acetone and in diethyl ether.

It dissolves in sodium hydroxide TS and in ammonia TS.

**Identification** (1)  
Dissolve 0.6 g of Mercaptopurine Hydrate in 6 mL of sodium hydroxide solution (3 in 100), and add slowly 0.5 mL of iodomethane with vigorous stirring. Stir well for 10 minutes, cool in an ice bath, and adjust the pH with acetic acid (31) to about 5. Collect the separated crystals by filtration, recrystallize from water, and dry at 120°C for 30 minutes: the crystals melt <2.60> between 218°C and 222°C (with decomposition).

(2) Determine the absorption spectrum of a solution of Mercaptopurine Hydrate in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity** (1)  
Clarity of solution—Dissolve 0.20 g of Mercaptopurine Hydrate in 10 mL of ammonia TS: the solution is clear.

(2) Sulfate <1.14>—Dissolve 50 mg of Mercaptopurine Hydrate in 10 mL of dilute hydrochloric acid, add 5 drops of barium chloride TS, and allow to stand for 5 minutes: no turbidity is produced.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Mercaptopurine Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).
(4) Hypoxanthine—Dissolve 50 mg of Mercaptopurine Hydrate in exactly 10 mL of a solution of ammonia solution (28) in methanol (1 in 10), and use this solution as the sample solution. Separately, dissolve 5.0 mg of hypoxanthine in a solution of ammonia solution (28) in methanol (1 in 10) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( \text{2.0}\). Spot 10 \( \mu \)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform, \( n \)-butyl formate and ammonia solution (28) \((8:6:4:1)\) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution observed at the same place as that from the standard solution, is not larger and not more intense than that from the standard solution.

(5) Phosphorus—Take 0.2 g of Mercaptopurine Hydrate in a crucible, add 2 mL of dilute sulfuric acid (3 in 7), then heat gently, slowly adding dropwise several 0.5-mL portions of nitric acid, until the liquid becomes colorless. Continue to heat until most of the liquid has evaporated, cool, and dissolve the residue in 10 mL of water. Transfer the solution to a 25-mL volumetric flask, wash the crucible with two 4-mL portions of water, combine the washings with the solution in the volumetric flask, and use this solution as the sample solution. Separately, dissolve 0.4396 g of potassium dihydrogenphosphate in water to make exactly 200 mL. To 2.0 mL of this solution add water to make exactly 100 mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, add 16 mL of water, and use this solution as the standard solution. To the sample solution and standard solution add 1 mL of diluted sulfuric acid (3 in 7), 0.5 mL of nitric acid, 0.75 mL of hexaammonium heptamolybdate TS, 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS and water to make 80 mL, then add 1 mL of nitric acid and water to make 100 mL, shake, and filter. Transfer 40 mL of the filtrate to a Nessler tube, add 6 mL of dilute nitric acid and water to make 50 mL, then add 1 mL of silver nitrate TS, mix well, and allow to stand for 5 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry \( \text{2.2} \), using water as the blank: the absorbance of the subsequent solution of the sample solution at 750 nm is not larger than that of the subsequent solution of the standard solution.

**Water** \( \text{2.4} \) 10.0 – 12.0% (0.2 g, volumetric titration, back titration).

**Residue on ignition** \( \text{2.4} \) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.25 g of Mercaptopurine Hydrate, dissolve in 90 mL of \( N, N \)-dimethylformamide, and titrate \( \text{2.5} \) with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Perform a blank determination with a mixture of 90 mL of \( N, N \)-dimethylformamide and 15 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS

\[ \frac{15.22 \text{ mg of } C_6H_7N_3S}{1 \text{ mL}} \]

**Containers and storage** Containers—Well-closed containers.

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### Mercurochrome

**Merbromin**

マーキュロクロム

Mercurochrome is a sodium salt of a mixture of brominated and mercurized fluoresceins.

When dried, it contains not less than 18.0% and not more than 22.4% of bromine (Br: 79.90), and not less than 22.4% and not more than 26.7% of mercury (Hg: 200.59).

**Description**

Mercurochrome occurs as blue-green to greenish red-brown scales or granules. It is odorless.

It is freely soluble in water, but sometimes leaves a small amount of insoluble matter. It is practically insoluble in ethanol (95) and in diethyl ether.

**Identification (1)** A solution of Mercurochrome (1 in 2000) shows a red color and a yellow-green fluorescence.

(2) To 5 mL of a solution of Mercurochrome (1 in 250) add 3 drops of dilute sulfuric acid: a reddish orange precipitate is produced.

(3) Heat 0.1 g of Mercurochrome with small crystals of iodine in a test tube: red crystals are sublimed on the upper part of the tube. If yellow crystals are produced, scratch with a glass rod: the color of the crystals changes to red.

(4) Place 0.1 g of Mercurochrome in a porcelain crucible, add 1 mL of a solution of sodium hydroxide (1 in 6), evaporate to dryness with stirring, and ignite. Dissolve the residue in 5 mL of water, acidify with hydrochloric acid, and shake with 3 drops of chloric acid and 2 mL of chloroform: a yellowish brown color develops in the chloroform layer.

**Purity (1)** Dyestuff—Dissolve 0.40 g of Mercurochrome in 20 mL of water, add 3 mL of dilute sulfuric acid, and filter: the filtrate has no more color than Matching Fluid C.

(2) Soluble halides—Dissolve 5.0 g of Mercurochrome in 80 mL of water, add 10 mL of dilute nitric acid and water to make 100 mL, shake, and filter. Transfer 40 mL of the filtrate to a Nessler tube, add 6 mL of dilute nitric acid and water to make 50 mL, then add 1 mL of silver nitrate TS, mix well, and allow to stand for 5 minutes protected from direct sunlight: no turbidity is produced, or even if produced, it is not more than that of the following control solution.

Control solution: To 0.25 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, then add 1 mL of silver nitrate TS, and proceed as directed above.

(3) Soluble mercury salts—To 5 mL of the filtrate obtained in (1) add 5 mL of water, and use this solution as the sample solution. Dissolve 40 mg of mercury (II) chloride, weighed accurately, in water to make 1000 mL, and add 3 mL of dilute sulfuric acid to 20 mL of this solution. To 5 mL of the solution add 5 mL of water, and use this as the control solution. Add 1 drop each of sodium sulfide TS to these solutions, and compare: the sample solution has no more color than the control solution.

(4) Insoluble mercury compounds—Dissolve 2.5 g of Mercurochrome in 50 mL of water, allow to stand for 24 hours, centrifuge, and wash the precipitate with small por-
tions of water until the last washing becomes colorless. Transfer the precipitate to a glass-stoppered flask, add exactly 5 mL of 0.05 mol/L iodine VS, allow to stand for 1 hour with frequent agitation, add 4.3 mL of 0.1 mol/L sodium thiosulfate VS dropwise with shaking, and add 1 mL of starch TS: a blue color develops.

**Loss on drying** $<2.41\%$ Not more than 5.0% (1 g, 105°C, 5 hours).

**Assay (1)** Mercuric—Weigh accurately about 0.6 g of Mercurochrome, previously powdered and dried, transfer to a porcelain crucible, add 2 g of potassium nitrate, 3 g of potassium carbonate and 3 g of anhydrous sodium carbonate, mix well, cover the surface of the mixture with 3 g of a mixture of equal amounts of potassium carbonate and anhydrous sodium carbonate, and ignite almost to fusion. Cool, dissolve the ignited mixture in 80 mL of warm water, acidify with nitric acid, and add exactly 25 mL of 0.1 mol/L silver nitrate VS. Shake well, and titrate with 0.1 mol/L sodium thiosulfate VS dropwise with shaking, and add 1 mL of starch TS. Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS = 10.03 mg of Hg

**Assay (2)** Bromine—Weigh accurately about 0.5 g of Mercurochrome, previously powdered and dried, in a porcelain crucible, add 2 g of potassium nitrate, 3 g of potassium carbonate and 3 g of anhydrous sodium carbonate, mix well, cover the surface of the mixture with 3 g of a mixture of equal amounts of potassium carbonate and anhydrous sodium carbonate, and ignite almost to fusion. Cool, dissolve the ignited mixture in 80 mL of warm water, acidify with nitric acid, and add exactly 25 mL of 0.1 mol/L silver nitrate VS. Shake well, and titrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS = 7.990 mg of Br

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

**Mercurochrome Solution**

**Merbromin Solution**

メルブロミン液

Mercurochrome Solution contains not less than 0.42 w/v% and not more than 0.56 w/v% of mercury (Hg: 200.59).

**Method of preparation**

<table>
<thead>
<tr>
<th>Mercurochrome</th>
<th>20 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified Water or Purified Water in Containers</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

Prepare by mixing the above ingredients.

**Description** Mercurochrome Solution is a dark red liquid.

**Identification (1)** To 1 mL of Mercurochrome Solution add 40 mL of water: the resulting solution shows a red color and a yellow-green fluorescence.

**Identification (2)** Dilute 1 mL of Mercurochrome Solution with 4 mL of water, and add 3 drops of dilute sulfuric acid: a red-orange precipitate is produced.

(3) Evaporate 5 mL of Mercurochrome Solution to dryness, and proceed with the residue as directed in the Identification (3) under Mercurochrome.

(4) To 5 mL of Mercurochrome Solution add 1 mL of a solution of sodium hydroxide (1 in 6), and proceed as directed in the Identification (4) under Mercurochrome.

**Purity** Dyestuff—To 20 mL of Mercurochrome Solution add 3 mL of dilute sulfuric acid, and filter: the filtrate has no more color than Matching Fluid C.

**Assay** Transfer exactly measured 30 mL of Mercurochrome Solution to an iodine flask, dilute with 20 mL of water, add 8 mL of acetic acid (31) and 20 mL of chloroform, and proceed as directed in the Assay (1) under Mercurochrome.

Each mL of 0.05 mol/L iodine VS = 10.03 mg of Hg

**Containers** Containers—Tight containers.

Storage—Light-resistant.

**Meropenem Hydrate**

メロペネム水和物

C$_{17}$H$_{25}$N$_{3}$O$_{5}$S$\cdot$3H$_{2}$O: 437.51

(4R,5S,6S)-3-{[(3S,5S)-5-(Dimethylcarbamoyl)pyrrolidin-3-ylsulfanyl]-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid trihydrate [119478-56-7]

Meropenem Hydrate contains not less than 980 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the anhydrous basis. The potency of Meropenem Hydrate is expressed as mass (potency) of meropenem (C$_{17}$H$_{25}$N$_{3}$O$_{5}$S: 383.46).

**Description** Meropenem Hydrate occurs as a white to light yellow crystalline powder.

It is sparingly soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

**Identification (1)** Dissolve 10 mg of Meropenem Hydrate in 2 mL of water, add 3 mL of hydroxylammonium chloride-ethanol TS, allow to stand for 5 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown color develops.

(2) Determine the absorption spectra of solutions of Meropenem Hydrate and Meropenem RS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $<2.24\%$, and compare the spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectra of Meropenem Hydrate and Meropenem RS as directed under Infrared Spectropho-
Meropenem Hydrate / Official Monographs

System suitability

— Determine the peak area ratio of the internal standard solution to the sample solution under the above operating conditions, and calculate the ratios, $Q_1$ and $Q_2$, of the area of meropenem obtained from 10 mL of this solution is equivalent to 16 to 24% of that from 10 $\mu$L of the standard solution.

System performance: Warm the sample solution at 60°C for 30 minutes. When the procedure is run with 10 $\mu$L of the sample solution under the above operating conditions, the ring-opened meropenem, meropenem and the dimer are eluted in this order, and the resolution between the peaks of the ring-opened meropenem and meropenem is not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of meropenem is not more than 1.5%.

Water

— Not less than 11.4% and not more than 13.4% (0.35 g, volumetric titration, direct titration).

Residue on ignition

— Not more than 0.1% (1 g).

Assay

— Weigh accurately an amount of Meropenem Hydrate and Meropenem RS, equivalent to about 50 mg (potency), add exactly 10 mL of the internal standard solution to dissolve, add triethylamine-phosphate buffer solution, pH 5.0 to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 $\mu$L of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, $Q_1$ and $Q_2$, of the peak area of meropenem to that of the internal standard.

\[ M_5 = \frac{M_5}{Q_1/Q_2} \times 1000 \]

$M_5$: Amount (mg) of Meropenem RS

Internal standard solution

— A solution of benzyl alcohol in triethylamine-phosphate buffer solution, pH 5.0 (1 in 300).

Operating conditions


Column: A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with octadecylsiloanized silica gel for liquid chromatography (5 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of triethylamine-phosphate buffer solution, pH 5.0 and methanol (5:1).

Flow rate: Adjust the flow rate so that the retention time of meropenem is about 7 minutes.

System suitability

— System performance: When the procedure is run with 5 $\mu$L of the standard solution under the above operating conditions, meropenem and the internal standard are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 6 times with 5 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of meropenem to that of the internal standard is not more than 1.0%.

Containers and storage

— Containers—Tight containers.
Meropenem for Injection

注射用メロペネム

Meropenem for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled potency of meropenem (C_{17}H_{25}N_{3}O_{5}S: 383.46).

Method of preparation Prepare as directed under Injection, with Meropenem Hydrate.

Description Meropenem for Injection occurs as a white to light yellow crystalline powder.

Identification Determine the infrared absorption spectrum of Meropenem for Injection as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25> it exhibits absorption at the wave numbers of about 3410 cm\(^{-1}\), 1750 cm\(^{-1}\), 1655 cm\(^{-1}\), 1583 cm\(^{-1}\) and 1391 cm\(^{-1}\).

\[ \text{pH} < 2.5 \] Dissolve an amount of Meropenem for Injection, equivalent to 0.25 g (potency) of Meropenem Hydrate according to the labeled amount, in 5 mL of water: the pH of the solution is between 7.3 and 8.3.

Purity (1) Clarity and color of solution—Dissolve an amount of Meropenem for Injection, equivalent to 1.0 g (potency) of Meropenem Hydrate according to the labeled amount, in 20 mL of water: the solution is clear and is not more intensely colored than the following matching fluid.

\[ \text{Matching fluid: To a mixture of 0.3 mL of Cobalt (II) Chloride CS and 1.2 mL of Iron (III) Chloride CS add 18.5 mL of diluted hydrochloric acid (1 in 40).} \]

\[ \text{pH < 2.5} \] Perform the test according to the Membrane filtration method: it meets the requirement.

\[ \text{Amount [mg (potency)] of meropenem (C}_{17}\text{H}_{25}\text{N}_{3}\text{O}_{5}\text{S}} = \frac{M_{5} \times Q_{7}}{Q_{6}} \]

\[ M_{5}: \text{Amount [mg (potency)] of Meropenem RS} \]

Internal standard solution—A solution of benzylo alcohol in triethylamine-phosphate buffer solution, pH 5.0 (1 in 300).

Operating conditions—Proceed as directed in the operating conditions in the Assay under Meropenem Hydrate.

System suitability—System performance: Proceed as directed in the system suitability in the Assay under Meropenem Hydrate.

System repeatability: When the test is repeated 6 times with 5 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of meropenem to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Mestranol

メストラノール

C_{21}H_{26}O_{2}: 310.43

3-Methoxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-17-ol [72-33-3]

Mestranol, when dried, contains not less than 97.0% and not more than 102.0% of C_{21}H_{25}O_{2}.

Description Mestranol occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in 1,4-dioxane, sparingly soluble in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

Identification (1) Dissolve 2 mg of Mestranol in 1 mL of a mixture of sulfuric acid and ethanol (99.5) (2:1): a red-purple color develops with a yellow-green fluorescence.

(2) Determine the absorption spectrum of a solution of Mestranol in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mestranol RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Mestranol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or
the spectrum of previously dried Mestranol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** \( <2.49 \) \( [\alpha]_{D}^{20} \): +2 – +8° (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

**Melting point** \( <2.60 \) 148 – 154°C

**Purity** (1) Heavy metals \( <1.07 \)—Proceed with 1.0 g of Mestranol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic \( <1.17 \)—Prepare the test solution with 1.0 g of Mestranol according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Mestranol in 20 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( <2.25 \). Spot 10 \( \mu L \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (99.5) (29:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly with a mixture of chloroform and ethanol (99.5) (29:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 5) on the plate, and heat at 105°C for 15 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** \( <2.41 \) Not more than 0.5% (0.5 g, 105°C, 3 hours).

**Residue on ignition** \( <2.44 \) Not more than 0.1% (0.5 g).

**Assay** Weigh accurately about 10 mg each of Mestranol and Mestranol RS, previously dried, dissolve in ethanol (99.5) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, \( A_{T} \) and \( A_{S} \), of the sample solution and the standard solution at 279 nm as directed under Ultraviolet-visible Spectrophotometry \( <2.24 \).

\[
\text{Amount (mg) of C}_{22}\text{H}_{32}\text{O}_{3} = M_{S} \times \frac{A_{T}}{A_{S}}
\]

where \( M_{S} \): Amount (mg) of Mestranol RS

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

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**Metenolone Acetate**

メテノロン酢酸エステル

C\(_{22}\)H\(_{32}\)O\(_{3}\): 344.49

1-Methyl-3-oxo-5α-androst-1-en-17β-yl acetate

[434-05-9]

Metenolone Acetate, when dried, contains not less than 97.0% and not more than 103.0% of C\(_{22}\)H\(_{32}\)O\(_{3}\).

**Description** Metenolone Acetate occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in acetone, in 1,4-dioxane and in chloroform, soluble in methanol and in ethanol (95), sparingly soluble in diethyl ether and in sesame oil, slightly soluble in hexane and in petroleum ether, and practically insoluble in water.

**Identification** (1) Dissolve 1 mg of Metenolone Acetate in 5 mL of a mixture of ethanol (95) and sulfuric acid (1:1), and heat for 30 minutes in a water bath: a red-brown color develops.

(2) To 10 mg of Metenolone Acetate add 0.5 mL of dilute sodium hydroxide-ethanol TS, and heat for 1 minute on a water bath. After cooling, add 0.5 mL of diluted sulfuric acid (1 in 2), and boil gently for 1 minute: the odor of ethyl acetate is perceptible.

(3) Dissolve 50 mg of Metenolone Acetate in 3 mL of methanol, add 0.3 mL of a solution of potassium carbonate (1 in 6), and boil for 2 hours under a reflux condenser. After cooling, add this solution gradually to 50 mL of cold water, and stir for 15 minutes. Filter the precipitate so obtained by suction through a glass filter (G4), wash with 10 mL of water, and dry at 105°C for 1 hour: it melts \( <2.60 \) between 157°C and 161°C.

(4) Determine the infrared absorption spectrum of Metenolone Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry \( <2.25 \), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** \( <2.49 \) \( [\alpha]_{D}^{20} \): +39 – +42° (after drying, 0.2 g, chloroform, 10 mL, 100 mm).

**Melting point** \( <2.60 \) 141 – 144°C

**Purity** (1) Clarity and color of solution—Dissolve 0.50 g of Metenolone Acetate in 10 mL of 1,4-dioxane: the solution is clear and colorless to pale yellow.

(2) Heavy metals \( <1.07 \)—Proceed with 2.0 g of Metenolone Acetate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 35 mg of Metenolone Acetate in 20 mL of chloroform, and use this solution as the
sample solution. Pipet 1 mL of the sample solution, dilute with chloroform to exactly 250 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.6D>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and cyclohexane (1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot should not be more intense than the spot from the standard solution.

**Loss on drying** <2.4F> Not more than 0.5% (0.5 g, 105°C, 3 hours).

**Residue on ignition** <2.4F> Not more than 0.1% (0.5 g).

**Assay** Weigh accurately about 10 mg of Metenolone Acetate, previously dried, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, and dilute with methanol to exactly 50 mL. Determine the absorbance, A, of this solution at the wavelength of maximum absorption at about 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.2G>.

\[
\text{Amount (mg) of C}_{27}\text{H}_{42}\text{O}_{3} = \frac{A}{391} \times 10,000
\]

Containers and storage Containers—Tight containers. Storage—Light-resistant.

**Metenolone Enanthate**

メテノロンエナント酸エステル

\[
\text{C}_{27}\text{H}_{42}\text{O}_{3} : 414.62
\]

1-Methyl-3-oxo-5α-androst-1-en-17β-yl heptanoate [303-42-4]

Metenolone Enanthate, when dried, contains not less than 97.0% and not more than 103.0% of C\(_{27}\)H\(_{42}\)O\(_{3}\).

**Description** Metenolone Enanthate occurs as white crystals or crystalline powder. It is odorless.

It is very soluble in ethanol (95), in acetone, in 1,4-dioxane and in chloroform, freely soluble in methanol, in ethyl acetate, in diethyl ether, in cyclohexane, in petroleum ether and in toluene, soluble in sesame oil, and practically insoluble in water.

**Identification** (1) Heat 1 mg of Metenolone Enanthate with 5 mL of a mixture of ethanol (95) and sulfuric acid (1:1) on a water bath for 30 minutes: a red-brown color develops.

(2) Dissolve 0.05 g of Metenolone Enanthate in 3 mL of methanol, add 0.3 mL of a solution of potassium carbonate (1 in 6), boil under a reflux condenser for 2 hours, cool, add slowly this solution to 50 mL of cold water, and stir for 15 minutes. Filter the produced precipitate by suction through a glass filter (G4), wash with water until the washings become neutral, and dry at 105°C for 1 hour: it melts 2.6D between 156°C and 162°C.

**Optical rotation** <2.4F> [α]\(_D\)^22D: +39 – +43° (after drying, 0.2 g, chloroform, 10 mL, 100 mm).

**Melting point** <2.6D> 67 – 72°C

**Purity** (1) Clarity and color of solution—Dissolve 0.5 g of Metenolone Enanthate in 10 mL of 1,4-dioxane: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Metenolone Enanthate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 20 mg of Metenolone Enanthate in exactly 10 mL of chloroform, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.6D>. Spot 10 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and cyclohexane (1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot does not appear.

**Loss on drying** <2.4F> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

**Residue on ignition** <2.4F> Not more than 0.1% (0.5 g).

**Assay** Weigh accurately about 0.1 g of Metenolone Enanthate, previously dried, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, and dilute with methanol to make exactly 100 mL. Pipet 10 mL of this solution, and dilute again with methanol to make exactly 100 mL. Determine the absorbance, A, of this solution at the wavelength of maximum absorption at about 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.2G>.

\[
\text{Amount (mg) of C}_{27}\text{H}_{42}\text{O}_{3} = \frac{A}{325} \times 100,000
\]

Containers and storage Containers—Tight containers. Storage—Light-resistant.

**Metenolone Enanthate Injection**

メテノロンエナント酸エステル注射液

Metenolone Enanthate Injection is an oily solution for injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of metenolone enanthate (C\(_{27}\)H\(_{42}\)O\(_{3}\); 414.62).

**Method of preparation** Prepare as directed under Injections, with Metenolone Enanthate.

**Description** Metenolone Enanthate Injection is a clear, pale yellow, oily liquid.

**Identification** (1) Measure a volume of Metenolone Enanthate Injection, equivalent to 0.1 g of Metenolone Enanthate according to the labeled amount, add 20 mL of
petroleum ether, and extract with three 20-mL portions of diluted acetic acid (100) (5 in 7). Combine the extracts, wash with 20 mL of petroleum ether, add 300 mL of cold water while cooling in an ice bath, and stir sufficiently. Filter the produced precipitate by suction through a glass filter (G4), wash with water until the last washing becomes neutral, and dry in a desiccator (in vacuum, phosphorus (V) oxide) for 6 hours. With this sample, proceed as directed in the Identification (1) under Metenolone Enanthate.

(2) Measure a volume of Metenolone Enanthate Injection, equivalent to 10 mg of Metenolone Enanthate according to the labeled amount, dissolve in 10 mL of chloroform, and use this solution as the sample solution. Separately dissolve 10 mg of metenolone enanthate in 10 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03>\). Spot 10 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with toluene to a distance of about 15 cm, and air-dry the plate. Again develop this plate with a mixture of 4-methyl-2-pentanone, 2-methoxyethanol, water and 20 mL of petroleum ether, add 300 mL of cold water while cooling in an ice bath, and stir sufficiently. Filter the produced precipitate by suction through a glass filter (G4), wash with water until the last washing becomes neutral, and dry in a desiccator (in vacuum, phosphorus (V) oxide) for 6 hours. With this sample, proceed as directed in the Identification (1) under Metenolone Enanthate.

Extractable volume \(<6.0>\) It meets the requirement.

Assay To an exactly measured volume of Metenolone Enanthate Injection, equivalent to about 0.1 g of metenolone enanthate \((C_27H_{42}O_3)\), add chloroform to make exactly 100 mL. Pipet 5 mL of this solution, add chloroform to make exactly 50 mL, and use this solution as the sample solution. Weigh accurately about 0.1 g of metenolone enanthate for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and prepare the standard solution in the same manner as directed for the preparation of the sample solution. Pipet 3 mL each of the sample solution and standard solution, and treat each solution as follows: add 10 mL of isoniazid TS, exactly measured, add methanol to make exactly 20 mL, and allow to stand for 60 minutes. Determine the absorbances, \(A_1\) and \(A_S\), of the solutions from the sample solution and standard solution, respectively, at 384 nm as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\), using a solution obtained by proceeding with 3 mL of chloroform as the blank.

\[
\text{Amount (mg) of metenolone enanthate (C}_2\text{H}_4\text{O}_3) = M_S \times \frac{A_1}{A_S}
\]

\(M_S\): Amount (mg) of metenolone enanthate for assay

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

### Metformin Hydrochloride

メトホルミン塩酸塩

C\(\text{H}_11\text{N}_5\text{HCl}\): 165.62

1,1-Dimethylbiguanide monohydrochloride [1115-70-4]

Metformin Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of \(\text{C}_4\text{H}_{11}\text{N}_5\text{HCl}\).

Description Metformin Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in water, sparingly soluble in acetic acid (100), and slightly soluble in ethanol (99.5).

Melting point: about 221°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Metformin Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Metformin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry \(<2.25>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Metformin Hydrochloride (1 in 50) responds to the Qualitative Tests \(<1.00>\) for chloride.

Purity (1) Heavy metals \(<1.07>\)—Proceed with 2.0 g of Metformin Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 2.5 g of Metformin Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution (1). Pipet 5 mL of the standard solution (1), add water to make exactly 10 mL, and use this solution as the standard solution (2). Separately, to 0.10 g of 1-cyanoguanidine add water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution (3). Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.03>\). Spot 10 \(\mu\)L each of the sample solution and standard solutions (1), (2) and (3) on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 4-methyl-2-pentanone, 2-methoxyethanol, water and acetic acid (100) (30:20:5:3) to a distance of about 10 cm, air-dry the plate, then dry at 105°C for 10 minutes. Spray evenly sodium pentacyanonitrosylferrate (III)-potassium hexacyanoferrate (III) TS on the plate: the spot other than the principal spot with the sample solution is not more intense than the principal spot with the standard solution (1), the num-
of them showing more intense than the spot with the
standard solution (2) is not more than two, and the spot with
the sample solution appeared at the position corresponding
to the spot with the standard solution (3) is not more intense
than the spot with the standard solution (3).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.4d> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g of Metformin Hydro-
chloride, previously dried, dissolve in 40 mL of acetic acid
(100), add 40 mL of acetic anhydride, and titrate
<2.50> with 0.05 mol/L perchloric acid VS (potentiometric titration).
Perform a blank determination in the same manner, and
make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 4.141 mg of C₄H₁₁N₅.HCl

Containers and storage Containers—Tight containers.

Metformin Hydrochloride Tablets
メトホルミン塩酸塩錠

Metformin Hydrochloride Tablets contain not less
than 95.0% and not more than 105.0% of the labeled
amount of metformin hydrochloride (C₄H₁₁N₅.HCl: 165.62).

Method of preparation Prepare as directed under Tablets,
with Metformin Hydrochloride.

Identification Shake an amount of powdered Metformin
Hydrochloride Tablets, equivalent to 250 mg of Metformin
Hydrochloride according to the labeled amount, with 25 mL
of 2-propanol, and filter. Evaporate the filtrate under
reduced pressure in a water bath at 40°C, and determine the
infrared absorption spectrum of the residue as directed in the
potassium chloride disk method under Infrared Spectrophot-
ometry <2.25>: it exhibits absorption at the wave numbers of
about 3370 cm⁻¹, 3160 cm⁻¹, 1627 cm⁻¹, 1569 cm⁻¹ and
1419 cm⁻¹.

Uniformity of dosage unit <6.02> It meets the requirement
of the Mass variation test.

Dissolution Being specified separately.

Assay Weigh accurately the mass of not less than 20 Met-
formin Hydrochloride Tablets, and powder. Weigh accur-
ately a portion of the powder, equivalent to about 0.15 g of
metformin hydrochloride (C₄H₁₁N₅.HCl), add 70 mL of a
mixture of water and acetonitrile (3:2), shake for 10 minutes,
add the mixture of water and acetonitrile (3:2) to make ex-
actly 100 mL, and filter through a membrane filter with a
pore size of not more than 0.45 μm. Discard the first 10 mL
of the filtrate, pipet 3 mL of the subsequent filtrate, add ex-
actly 3 mL of the internal standard solution and the mixture
of water and acetonitrile (3:2) to make 50 mL, and use this
solution as the sample solution. Separately, weigh accurately
about 0.15 g of metformin hydrochloride for assay, previ-
ously dried at 105°C for 3 hours, and dissolve in the mixture
of water and acetonitrile (3:2) to make exactly 100 mL. Pipet
3 mL of this solution, add exactly 3 mL of the internal stand-
ard solution and the mixture of water and acetonitrile (3:2)
to make 50 mL, and use this solution as the standard solu-
tion. Perform the test with 5 μL each of the sample solution
and standard solution as directed under Liquid Chromatog-
raphy <2.01> according to the following conditions, and cal-
culate the ratios, Q₁ and Q₂, of the peak area of metformin
to that of the internal standard.

Amount (mg) of metformin hydrochloride (C₄H₁₁N₅.HCl)
= Mₛ × Q₁/Q₂

Mₛ: Amount (mg) of metformin hydrochloride for assay

Internal standard solution—Dissolve 0.3 g of isobutyl para-
hydroxybenzoate in 100 mL of the mixture of water and
acetonitrile (3:2).

Operating conditions—
Detector: An ultraviolet absorption photometer (wave-
length: 235 nm).
Column: A stainless steel column 4.6 mm in inside diam-
eter and 15 cm in length, packed with octadeclsilanized silica
gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about
40°C.

Mobile phase: Dissolve 0.8 g of sodium lauryl sulfate in
620 mL of diluted phosphoric acid (1 in 2500), and add 380
mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time
of metformin is about 10 minutes.

System suitability—
System performance: When the procedure is run with 5 μL
of the standard solution under the above operating condi-
tions, metformin and the internal standard are eluted in this
order with the resolution between these peaks being not less
than 6.

System repeatability: When the test is repeated 6 times
with 5 μL of the standard solution under the above operating
conditions, the relative standard deviation of the ratio of the
peak area of metformin to that of the internal standard is
not more than 1.0%.

Containers and storage Containers—Well-closed contain-
ers.

Methamphetamine Hydrochloride
メタンフェタミン塩酸塩

Methamphetamine Hydrochloride, when dried, con-
tains not less than 98.5% of C₁₀H₁₅N.HCl.

Description Methamphetamine Hydrochloride occurs as
colorless crystals or a white, crystalline powder. It is odor-
less.

It is freely soluble in water, in ethanol (95) and in chlo-
roform, and practically insoluble in diethyl ether.

The pH of a solution of Methamphetamine Hydrochloride (1 in 10) is between 5.0 and 6.0.

**Identification**

1. To 5 mL of a solution of Methamphetamine Hydrochloride (1 in 100) add 0.5 mL of hydrogen hexachloroplatinate (IV) TS: an orange-yellow, crystalline precipitate is produced.
2. To 5 mL of a solution of Methamphetamine Hydrochloride (1 in 100) add 0.5 mL of 2,4,6-trinitrophenol TS: a yellow, crystalline precipitate is produced.
3. A solution of Methamphetamine Hydrochloride (1 in 20) responds to the Qualitative Tests for chloride.

**Optical rotation**

< 19° (after drying, 0.2 g, water, 10 mL, 100 mm).

**Melting point**

< 171 – 175°C

**Purity**

(i) Acidity or alkalinity—Dissolve 2.0 g of Methamphetamine Hydrochloride in 40 mL of freshly boiled and cooled water, add 2 drops of methyl red TS, and use this solution as the sample solution.

(ii) To 20 mL of the sample solution add 0.20 mL of 0.01 mol/L sulfuric acid VS: a red color develops.

(iii) To 20 mL of the sample solution add 0.20 mL of 0.02 mol/L sodium hydroxide VS: a yellow color develops.

(ii) Sulfate

Dissolve 0.05 g of Methamphetamine Hydrochloride in 40 mL of water, add 1 mL of dilute hydrochloric acid and 1 mL of barium chloride TS, and allow to stand for 10 minutes: the solution remains unchanged.

**Loss on drying**

Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition**

Not more than 0.1% (1 g).

**Assay**

Weigh accurately about 0.4 g of Methamphetamine Hydrochloride, previously dried, and dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100): (7:3). Titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 18.57 mg of C₅H₁₁NO₂S.

**Containers and storage**

Containers—Tight containers.

Storage—Light-resistant.

**Description**

L-Methionine occurs as white crystals or crystalline powder. It has a characteristic odor.

It is freely soluble in formic acid, soluble in water, and very slightly soluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

**Identification**

Determine the infrared absorption spectrum of L-Methionine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation**

< 24° [α]21D = +16 – +19° (after drying, 0.5 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

**pH**

Dissolve 0.5 g of L-Methionine in 20 mL of water: the pH of this solution is between 5.2 and 6.2.

**Purity**

(i) Clarity and color of solution—Dissolve 0.5 g of L-Methionine in 20 mL of water: the solution is clear and colorless.

(ii) Chloride

Dissolve 0.5 g of L-Methionine in 20 mL of water, and add 6 mL of dilute nitric acid and water to make 40 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS, 6 mL of dilute nitric acid and water to make 40 mL. In this test, to the test solution and the control solution add 10 mL each of silver nitrate TS (not more than 0.021%).

(iii) Sulfate

—Dissolve 0.05 g of L-Methionine in 40 mL of water, and add 1 mL of dilute hydrochloric acid and 1 mL of barium chloride TS, and allow to stand for 10 minutes: the solution remains unchanged.

**Heavy metals**

—Dissolve 1.0 g of L-Methionine in 40 mL of water and 2 mL of dilute acetic acid, dissolve by warming, cool, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

**Arsenic**

—Transfer 1.0 g of L-Methionine to a 100-mL decomposition flask, add 5 mL of nitric acid and 2 mL of sulfuric acid, put a small funnel on the mouth of the flask, and heat carefully until white fumes are evolved. After cooling, add two 2-mL portions of nitric acid, heat, and add 2-mL portions of hydrogen peroxide (30) several times, and heat until the solution becomes colorless or pale yellow. After cooling, add 2 mL of saturated ammonium oxalate monohydrate solution, and heat again until white fumes are evolved. After cooling, add water to make 5 mL, and perform the test with this solution as the test solution (not more than 2 ppm).

**Related substances**—Dissolve 0.10 g of L-Methionine in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography: spot 5 mL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. After air-drying, immediately develop the plate with a mixture of 1-butanol, water.
and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** 2.41% Not more than 0.30% (1 g, 105°C, 3 hours).

**Residue on ignition** 2.44% Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.15 g of l-Methionine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 14.92 mg of C₇H₁₇NO₂S

**Containers and storage** Containers—Tight containers.

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**Methotrexate**

メトトレキサート

![Methotrexate molecule](image)

C₂₀H₂₂N₈O₅: 454.44

N-{4-[[2,4-Diaminopteridin-6-ylmethyl](methyl)amino]benzoyl]-L-glutamic acid [59-05-2]

Methotrexate is a mixture of 4-amino-10-methylfolic acid and closely related compounds.

It contains not less than 94.0% and not more than 102.0% of C₂₀H₂₂N₈O₅, calculated on the anhydrous basis.

**Description** Methotrexate occurs as a yellow-brown, crystalline powder.

It is slightly soluble in pyridine, and practically insoluble in water, in acetonitrile, in ethanol (95) and in diethyl ether.

It dissolves in dilute sodium hydroxide TS and in dilute sodium carbonate TS.

It is gradually affected by light.

**Identification** (1) Dissolve 1 mg of Methotrexate in 100 mL of 0.1 mol/L hydrochloric acid TS. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry 2.24%, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methotrexate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Methotrexate as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25%, and compare the spectrum with the Reference Spectrum or the spectrum of Methotrexate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Water** 2.48% Take 5 mL of pyridine for water determination and 20 mL of methanol for Karl Fischer method in a dried titration flask, and titrate with water determination TS until the end point. Weigh accurately about 0.2 g of Methotrexate, immediately place in the titration flask, and add a known excess volume of Karl Fischer TS. Mix well for 30 minutes, and perform the test: the water content is not more than 12.0%.

**Residue on ignition** 2.44% Not more than 0.1% (0.5 g).

**Assay** Weigh accurately about 25 mg each of Methotrexate and Methotrexate RS, dissolve in the mobile phase to make exactly 250 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μL each of these solutions as directed under Liquid Chromatography 2.07% according to the following conditions, and determine the peak areas, Aₜ and Aₛ, of methotrexate in each solution.

Amount (mg) of C₂₀H₂₂N₈O₅ = Mₛ × Aₜ/Aₛ

Mₛ: Amount (mg) of Methotrexate RS, calculated on the anhydrous basis

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 302 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of disodium hydrogen phosphate-citric acid buffer solution, pH 6.0 and acetonitrile (89:11).

Flow rate: Adjust the flow rate so that the retention time of methotrexate is about 8 minutes.

**System suitability**—

System performance: Dissolve 10 mg each of Methotrexate and folic acid in 100 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, folic acid and methotrexate are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methotrexate is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

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**Methotrexate Capsules**

メトトレキサートカプセル

Methotrexate Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of methotrexate (C₂₀H₂₂N₈O₅: 454.44).

**Method of preparation** Prepare as directed under Capsules, with Methotrexate.
Identification To an amount of the content of Methotrexate Capsules, equivalent to 2 mg of Methotrexate according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake, and filter. To 10 mL of the filtrate add 0.1 mol/L hydrochloric acid TS to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry $\lambda_{244}$: it exhibits maxima between 240 nm and 244 nm and between 304 nm and 308 nm.

Uniformity of dosage units $\lfloor 0.02 \rfloor$ Perform the test according to the following method: it meets the requirement of the Content uniformity test. To the content of 1 capsule of Methotrexate Capsules add $3V/5$ mL of the mobile phase, agitate with the aid of ultrasonic waves for 15 minutes, then shake for 25 minutes, and add the mobile phase to make exactly $V$ mL so that each mL contains about 20 $\mu$g of methotrexate (C$_{20}$H$_{22}$N$_8$O$_5$). Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Methotrexate RS (separately determine the water $< 2.48$ in the same manner as Methotrexate), and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 $\mu$L of each of the sample solution and standard solution as directed under Liquid Chromatography $\cup 0.1 \times \gamma$ according to the following conditions, and determine the peak areas, $A_T$ and $A_S$, of methotrexate of both solutions.

\[
\text{Dissolution rate (\% with respect to the labeled amount of methotrexate (C}_{20}\text{H}_{22}\text{N}_8\text{O}_5)} = \frac{M_S \times A_T}{A_S \times V'/V \times 1/C \times 18}
\]

$M_S$: Amount (mg) of Methotrexate RS, calculated on the anhydrous basis

$C$: Labeled amount (mg) of methotrexate (C$_{20}$H$_{22}$N$_8$O$_5$) in 1 capsule

Operating conditions— Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 50 $\mu$L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of methotrexate are not less than 3500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methotrexate is not more than 1.0%.

Assay Accurately weigh the mass of not less than 20 Methotrexate Capsules, take out all of the content, and accurately weigh the mass of the empty capsules. Powder the content, weigh accurately a portion of the powder, equivalent to about 10 mg of methotrexate (C$_{20}$H$_{22}$N$_8$O$_5$), add 60 mL of the mobile phase, shake for 25 minutes, and add the mobile phase to make exactly 100 mL. Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Methotrexate RS (separately determine the water $< 2.48$ in the same manner as Methotrexate), and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 20 $\mu$L of each of the sample solution and standard solution as directed under Liquid Chromatography $\cup 0.1 \times \gamma$, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of methotrexate to that of the internal standard.

\[
\text{Amount (mg) of methotrexate (C}_{20}\text{H}_{22}\text{N}_8\text{O}_5)} = M_S \times Q_T/Q_S
\]

$M_S$: Amount (mg) of Methotrexate RS, calculated on the anhydrous basis

Internal standard solution—A solution of 4-nitrophenol in methanol (1 in 10,000).

Operating conditions— Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methotrexate to that of the internal standard is not more than 1.0%.

Dissolution $\lfloor 0.1 \rfloor$ When the test is performed at 50 revolutions per minute according to the Paddle method using a sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Methotrexate Capsules is not less than 85%.

Start the test with 1 capsule of Methotrexate Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 $\mu$m. Discard the first 10 mL of the filtrate, pipet $V$ mL of the subsequent filtrate, add water to make exactly $V'$ mL so that each mL contains about 2.2 $\mu$g of methotrexate (C$_{20}$H$_{22}$N$_8$O$_5$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Methotrexate RS (separately determine the water $< 2.48$ in the same manner as Methotrexate), and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography $\cup 0.1 \times \gamma$ according to the following conditions, and determine the peak areas, $A_T$ and $A_S$, of methotrexate of both solutions.

\[
\text{Dissolution rate (\% with respect to the labeled amount of methotrexate (C}_{20}\text{H}_{22}\text{N}_8\text{O}_5)} = \frac{M_S \times A_T}{A_S \times V'/V \times 1/C \times 18}
\]

$M_S$: Amount (mg) of Methotrexate RS, calculated on the anhydrous basis

$C$: Labeled amount (mg) of methotrexate (C$_{20}$H$_{22}$N$_8$O$_5$) in 1 capsule

Operating conditions— Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 50 $\mu$L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of methotrexate are not less than 3500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methotrexate is not more than 1.0%.

Assay Accurately weigh the mass of not less than 20 Methotrexate Capsules, take out all of the content, and accurately weigh the mass of the empty capsules. Powder the content, weigh accurately a portion of the powder, equivalent to about 10 mg of methotrexate (C$_{20}$H$_{22}$N$_8$O$_5$), add 60 mL of the mobile phase, shake for 25 minutes, and add the mobile phase to make exactly 100 mL. Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Methotrexate RS (separately determine the water $< 2.48$ in the same manner as Methotrexate), and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 $\mu$L of each of the sample solution and standard solution as directed under Liquid Chromatography $\cup 0.1 \times \gamma$, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of methotrexate to that of the internal standard.

\[
\text{Amount (mg) of methotrexate (C}_{20}\text{H}_{22}\text{N}_8\text{O}_5)} = M_S \times Q_T/Q_S
\]

$M_S$: Amount (mg) of Methotrexate RS, calculated on the anhydrous basis

Internal standard solution—A solution of 4-nitrophenol in methanol (1 in 10,000).
methanol (1 in 10,000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 302 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: To 250 mL of 0.2 mol/L potassium dihydrogen phosphate TS add 28.5 mL of 0.2 mol/L sodium hydroxide TS and water to make 1000 mL. To 890 mL of this solution add 110 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of methotrexate is about 6 minutes.

System suitability—
System performance: Dissolve 10 mg each of methotrexate and folic acid in 100 mL of the mobile phase. To 2 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, folic acid and methotrexate are eluted in this order with the resolution between these peaks being not less than 8.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methotrexate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Methoxsalen
メトキサレン

C_{12}H_{8}O_{4}: 216.19
9-Methoxy-7H-furo[3,2-g]chromen-7-one [298-81-7]

Methoxsalen contains not less than 98.0% and not more than 102.0% of C_{12}H_{8}O_{4}, calculated on the anhydrous basis.

Description Methoxsalen occurs as white to pale yellow crystals or crystalline powder. It is odorless and tasteless.
It is freely soluble in chloroform, slightly soluble in methanol, in ethanol (95) and in diethyl ether, and practically insoluble in water.

Identification (1) To 10 mg of Methoxsalen add 5 mL of dilute nitric acid, and heat; a yellow color develops. Make this solution alkaline with a solution of sodium hydroxide (2 in 5); the color changes to red-brown.
(2) To 10 mg of Methoxsalen add 5 mL of sulfuric acid, and shake; a yellow color develops.
(3) Determine the absorption spectrum of a solution of Methoxsalen in ethanol (95) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methoxsalen RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point 2.60° 145 – 149°C

Purity (1) Heavy metals 1.07—Proceed with 1.0 g of Methoxsalen according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).
(2) Arsenic 1.11—Prepare the test solution with 1.0 g of Methoxsalen according to Method 3, and perform the test (not more than 2 ppm).
(3) Related substances—Dissolve 50 mg of Methoxsalen in 10 mL of chloroform, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add chloroform to make exactly 50 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.05. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, hexane and ethyl acetate (40:10:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm); the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water 2.46 Not more than 0.5% (1 g, volumetric titration, direct titration).

Residue on ignition 2.44 Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Methoxsalen and Methoxsalen RS, and dissolve each in ethanol (95) to make exactly 100 mL. Pipet 2 mL each of these solutions, and dilute each with ethanol (95) to make exactly 25 mL. Pipet 10 mL each of these solutions, and dilute each again with ethanol (95) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, A_{T} and A_{S}, of the sample solution and the standard solution at 300 nm as directed under Ultraviolet-visible Spectrophotometry 2.24:

\[
\text{Amount (mg) of } C_{12}H_{8}O_{4} = M_{S} \times A_{T} / A_{S}
\]

M_{S}: Amount (mg) of Methoxsalen RS, calculated on the anhydrous basis.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.
Methylbenactyzium Bromide

Methylbenactyzium Bromide, when dried, contains not less than 99.0% of C_{21}H_{28}BrNO_{3}.

**Description** Methylbenactyzium Bromide occurs as white crystals or crystalline powder. It is odorless, and has an extremely bitter taste.

It is freely soluble in water and in acetic acid (100), soluble in ethanol (95), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Methylbenactyzium Bromide (1 in 100) is between 5.0 and 6.0.

**Identification** (1) Shake 0.5 mL of a solution of Methylbenactyzium Bromide (1 in 100) with 5 mL of phosphate buffer solution, pH 7.0, to 2 to 3 drops of bromothymol blue TS and 5 mL of chloroform: a yellow color develops in the chloroform layer.

(2) To about 1 g of Methylbenactyzium Bromide add 5 mL of water and 10 mL of sodium hydroxide TS, allow to stand for 5 minutes, add 5 mL of dilute hydrochloric acid, collect the precipitate, wash well with water, recrystallize from a mixture of water and ethanol (95) (10:3), and dry at 90°C for 1 hour: the crystals melt <2.60> between 145°C and 150°C. Continue the heating up to about 200°C: a red color develops.

(3) Add 2 mL of dilute nitric acid to 5 mL of a solution of Methylbenactyzium Bromide (1 in 10): the solution responds to the Qualitative Tests <1.09> (1) for bromide.

**Melting point** <2.60> 168 - 172°C

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Methylbenactyzium Bromide in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 0.5 g of Methylbenactyzium Bromide. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Methylbenactyzium Bromide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**Loss on drying** <2.41> Not more than 0.5% (2 g, 105°C, 2 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Methylbenactyzium Bromide, previously dried, and dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (4:1). Titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 42.24 mg of C_{21}H_{28}BrNO_{3}

**Containers and storage** Containers—Tight containers.

---

**Methylcellulose**

Cellulose, methyl ether

Methylcellulose occurs as a white to yellowish white, powder or granules.

It is practically insoluble in ethanol (99.5).

Methylcellulose swells, when water is added, and forms a clear or slightly turbid, viscous liquid.

**Description** Methylcellulose occurs as a white to yellowish white, powder or granules.

It is practically insoluble in ethanol (99.5).

**Identification** (1) Disperse evenly 1.0 g of Methylcellulose over the surface of 100 mL of water in a beaker, while gently tapping the top of the container, if necessary, and allow the beaker to stand: it aggregates on the surface of water.

(2) Add 1.0 g of Methylcellulose to 100 mL of hot water, and stir: it becomes a suspension. Cool the suspension to 5°C, and stir: the resulting liquid is a clear or a slightly cloudy, viscous fluid.

(3) To 0.1 mL of the viscous fluid obtained in (2) add 9 mL of dilute sulfuric acid (9 in 10), stir, heat in a water bath for exactly 3 minutes, and immediately cool in ice water. Add carefully 0.6 mL of ninhydrin TS, stir, and allow to stand at 25°C: the solution shows a light red color, and it does not change to purple color within 100 minutes.

(4) Pour and spread out 2 to 3 mL of the viscous fluid obtained in (2) onto a glass plate, and allow the water to evaporate: a transparent film results.

(5) Pipet 50 mL of water, add exactly 50 mL of the viscous fluid obtained in (2), and warm to rise the temperature at a rate of 2 to 5°C per minute while stirring: the temperature, when a white turbidity of the solution starts to increase, is not less than 50°C.

**Viscosity** <2.53>

(1) Method I: Apply to Methylcellulose having a labeled viscosity of less than 600 mPa·s. Put exactly an amount of Methylcellulose, equivalent to 4.000 g on the dried basis, in a tared, wide-mouth bottle, add hot water to make 200.0 g, stopper the bottle, stir by mechanical means at 350- to 450-
revolutions per minute for 10 to 20 minutes to get a homogeneous dispersion. If necessary, take off the sample attached on the walls of the bottle, put them in the dispersed solution, and dissolve by continuing the stirring in a water bath not exceeding 5 °C for 20 to 40 minutes. Add cooled water, if necessary, to make 200.0 g, and use this solution as the sample solution. Centrifuge the solution if necessary to expel any entrapped air bubbles. Perform the test with the sample solution at 20 ± 0.1 °C as directed in Method I under Viscosity Determination: not less than 80% and not more than 120% of the labeled viscosity.

(ii) Method II: Apply to Methylcellulose having a labeled viscosity of not less than 600 mPa·s. Put exactly an amount of Methylcellulose, equivalent to 10.00 g on the dried basis, in a tared, wide-mouth bottle, add hot water to make 500.0 g, stopper the bottle, and prepare the sample solution in the same manner as directed in Method I. Perform the test with the sample solution at 20 ± 0.1 °C as directed in Method II (2) under Viscosity Determination, using a single cylinder-type rotational viscometer, according to the following operating conditions: not less than 75% and not more than 140% of the labeled viscosity.

**Operating conditions—**

Apparatus: Brookfield type viscometer LV model.

<table>
<thead>
<tr>
<th>Labeled viscosity (mPa·s)</th>
<th>Rotor No.</th>
<th>Rotation frequency /min</th>
<th>Conversion factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not less than 600 and less than 1400</td>
<td>3</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>n 1400</td>
<td>n 3500</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>n 3500</td>
<td>n 9500</td>
<td>4</td>
<td>60</td>
</tr>
<tr>
<td>n 9500</td>
<td>n 99,500</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>n 99,500</td>
<td></td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

Procedure of apparatus: Read value after 2 minutes of rotation, and stop the rotation for 2 minutes. Repeat this procedure more two times, and average three observed values.

**pH <2.54>** Allow the sample solution obtained in the Viscosity to stand at 20 ± 2 °C for 5 minutes: the pH of the solution thus obtained is between 5.0 and 8.0.

**Purity** Heavy metals—Put 1.0 g of Methylcellulose in a 100-mL kjeldahl flask, add a sufficient amount of a mixture of nitric acid and sulfuric acid (5:4) to wet the sample, and heat gently. Repeat this procedure until use totally 18 mL of the mixture of nitric acid and sulfuric acid. Then boil gently until the solution changes to black. After cooling, add 2 mL of nitric acid, and heat until the solution changes to black. Repeat this procedure until the solution no longer changes to black, and heat strongly until dense white fumes are evolved. After cooling, add 5 mL of water, boil gently until dense white fumes are evolved, then heat until the volume of the solution becomes to 2 to 3 mL. After cooling, if the solution reveals yellow color by addition of 5 mL of water, add 1 mL of hydrogen peroxide (30), and heat until the volume of the solution becomes to 2 to 3 mL. After cooling, dilute the solution with 2 to 3 mL of water, transfer to a Nessler tube, add water to make 25 mL, and use this solution as the test solution. Separately, put 2.0 mL of Standard Lead Solution in a 100-mL kjeldahl flask, add 18 mL of the mixture of nitric acid and sulfuric acid (5:4) and an amount of nitric acid equal to that used for preparation of the test solution, and heat until white fumes are evolved. After cooling, add 10 mL of water. In the case where hydrogen peroxide (30) is added for the preparation of the test solution, add the same amount of hydrogen peroxide (30), then proceed in the same manner for preparation of the test solution, and use so obtained solution as the control solution. Adjust the test solution and the control solution to pH 3.0 to 4.0 with ammonia solution (28), and add water to make 40 mL, respectively. To these solutions add 1.2 mL of thioacetamide-alkaline glycerin TS, 2 mL of acetate buffer solution, pH 3.5 and water to make 50 mL, separately. After allowing to stand for 5 minutes, observe vertically both tubes on a white background: the color obtained with the test solution is not more intense than that with the control solution (not more than 20 ppm).

**Loss on drying <2.41>** Not more than 5.0% (1 g, 105 °C, 1 hour).

**Residue on ignition <2.44>** Not more than 1.5% (1 g).

**Assay**

(i) Apparatus—Reaction bottle: A 5-mL pressure-tight glass vial, having 20 mm in outside diameter and 50 mm in height, the neck 20 mm in outside diameter and 13 mm in inside diameter, equipped with a septum of butyl-rubber processed the surface with fluoroplastics, which can be fixed tightly to vial with aluminum cap, or equivalent.

Heater: A shape-squared aluminum block, having holes 20 mm in diameter and 32 mm in depth, adopted to the reaction bottle. Capable of stirring the content of the reaction bottle by means of magnetic stirrer or of reciprocal shaker about 100 times per minute.

(ii) Procedure—Weigh accurately about 65 mg of Methylcellulose, transfer to the reaction bottle, add 0.06 to 0.10 g of adpic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid, stopper the bottle immediately, and weigh accurately. Stir or shake for 60 minutes while heating so that the temperature of the bottle content is 130 ± 2 °C. In the case where the stirrer or shaker is not available, heat for 30 minutes with repeated shaking at 5-minute intervals by hand, and continue heating for an additional 30 minutes. Allow the bottle to cool, and again weigh accurately. If the mass loss is less than 0.50% or there is no evidence of a leak, use the upper layer of the mixture as the sample solution. Separately, put 0.06 to 0.10 g of adpic acid in a reaction bottle, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid, stopper the bottle immediately, and weigh accurately. Add 45 μL of iodomethane for assay through the septum using micro-syringe, weigh accurately, stir thoroughly, and use the upper layer of the mixture as the standard solution. Perform the test with 1 to 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q1/Q0, of the peak area of iodomethane to that of the internal standard.

Content (%) of methoxy group (CH₃O)

\[ M_s/M = Q_1/Q_0 \times 21.86 \]

Mₘ: Amount (mg) of iodomethane in the standard solution

M: Amount (mg) of sample, calculated on the dried basis
Internal standard solution—A solution of n-octane in o-xylene (3 in 100).

Operating conditions—
Detector: A thermal conductivity detector or hydrogen flame-ionization detector.
Column: A glass column 3 - 4 mm in inside diameter and 1.8 - 3 m in length, packed with siliceous earth for gas chromatography, 125 to 150 μm in diameter, coated with methyl silicone polymer at the ratio of 10 – 20%.
Column temperature: A constant temperature of about 100°C.
Carrier gas: Helium for thermal conductivity detector, or Helium or Nitrogen for hydrogen, flame-ionization detector.
Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 10 minutes.

System suitability—
System performance: When the procedure is run with 1 – 2 μL of the standard solution under the above operating conditions, iodomethane and the internal standard are eluted in this order, with complete separation of these peaks.

*Containers and storage Containers—Well-closed containers.

**Methyldopa Hydrate**

メチルドパ水和物

![Structural formula of Methyldopa Hydrate](image)

C₁₀H₁₃NO₄·1½H₂O: 238.24
(2S)-2-Amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid sesquihydrate
[41372-08-1]

Methyldopa Hydrate contains not less than 98.0% of methyldopa (C₁₀H₁₃NO₄·211.21), calculated on the anhydrous basis.

**Description** Methyldopa Hydrate occurs as a white to pale grayish white, crystalline powder.
It is slightly soluble in water, in methanol and in acetic acid (100), very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.
It dissolves in dilute hydrochloric acid.

**Identification** (1) To 10 mg of Methyldopa Hydrate add 3 drops of ninhydrin TS, and heat in a water bath for 3 minutes: a purple color develops.
(2) Determine the absorption spectrum of a solution of Methyldopa Hydrate in 0.1 mol/L hydrochloric acid TS (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <2.44>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methyldopa RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.
(3) Determine the infrared absorption spectrum of Methyldopa Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Methyldopa RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> [α]D: −25 – −28° (calculated on the anhydrous basis, 1 g, aluminum (III) chloride TS, 20 mL, 100 mm).

**Purity** (1) Acidity—Shake 1.0 g of Methyldopa Hydrate with 100 mL of freshly boiled and cooled water, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 2 drops of methyl red TS: a yellow color develops.
(2) Chloride <1.07>—Perform the test with 0.5 g of Methyldopa Hydrate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).
(3) Heavy metals <1.07>—Proceed with 2.0 g of Methyldopa Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).
(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Methyldopa Hydrate in 5 mL of dilute hydrochloric acid, and perform the test (not more than 2 ppm).
(5) 3-O-Methyldopa—Dissolve 0.10 g of Methyldopa Hydrate in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 5 mg of 3-O-methyldopa for thin-layer chromatography in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 20 μL each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (13:5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-nitroaniline-sodium nitrite TS on the plate, and air-dry the plate, then spray evenly a solution of sodium carbonate decahydrate (1 in 4) on the plate: the spot from the sample solution corresponding to that from the standard solution is not more intense than the spot from the standard solution.

**Water** <2.48> 10.0 – 13.0% (0.2 g, volumetric titration, direct titration).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Methyldopa Hydrate, dissolve in 80 mL of acetic acid (100), and titrate <2.59> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 to 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 21.12 mg of C₁₀H₁₃NO₄

*Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.*
Methyldopa Tablets

メチルドパ錠

Methyldopa Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of methyldopa (C₁₀H₁₃NO₄; 211.21).

Method of preparation Prepare as directed under Tablets, with Methyldopa Hydrate.

Identification (1) To a quantity of powdered Methyldopa Tablets, equivalent to 0.1 g of Methyldopa Hydrate according to the labeled amount, add 10 mL of water, and heat in a water bath for 5 minutes with occasional shaking. After cooling, centrifuge for 5 minutes at 2000 rotations per minute, apply 1 drop of the supernatant solution to a filter paper, and dry with warm air. Place 1 drop of ninhydrin TS over the spot, and heat for 5 minutes at 100°C: a purple color develops.

(2) To 0.5 mL of the supernatant liquid obtained in the Identification (1) add 2 mL of 0.05 mol/L sulfuric acid TS, 2 mL of iron (II) tartrate TS and 4 drops of ammonia TS, and shake well: a deep purple color develops.

(3) To 0.7 mL of the supernatant liquid obtained in the Identification (1) add 0.1 mol/L hydrochloric acid TS to make 20 mL. To 10 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2,24>: it exhibits a maximum between 277 nm and 283 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Methyldopa Tablets add 50 mL of 0.05 mol/L sulfuric acid TS, shake for 15 minutes, then add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet V mL of the subsequent filtrate equivalent to about 5 mg of methyldopa (C₁₀H₁₃NO₄), add exactly 5 mL of iron (II) tartrate TS, then add ammonia-ammonium acetate buffer solution, pH 8.5, to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.11 g of Methyldopa RS (previous dried at 125°C for 2 hours), and dissolve in 0.05 mol/L sulfuric acid TS, shake thoroughly for 15 minutes, and add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter through a dry filter paper. Discard the first 20 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 0.11 g of Methyldopa RS (previous dried at 125°C for 2 hours), and determine the loss on drying <2,41> at 125°C for 2 hours, and determine the loss on drying <2,41>, dissolve in 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, add exactly 5 mL of iron (II) tartrate TS, and add ammonia-ammonium acetate buffer solution, pH 8.5, to make exactly 100 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2,24>, using a solution prepared with 5 mL of 0.05 mol/L sulfuric acid TS in the same manner, as the blank. Determine the absorbances, Aₜ and Aₛ, of the subsequent solutions of the sample solution and the standard solution at 520 nm, respectively.

\[
\text{Amount (mg) of methyldopa (C₁₀H₁₃NO₄)} = Mₛ \times Aₜ/ Aₛ \times 5/ V
\]

Mₛ: Amount (mg) of Methyldopa RS, calculated on the dried basis

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 60 minutes of Methyldopa Tablets is not less than 75%.

Start the test with 1 tablet of Methyldopa Tablets, withdraw not less than 30 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 25 μg of methyldopa (C₁₀H₁₃NO₄) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 56 mg of methyldopa for assay (separately determine the loss on drying <2,41> at 125°C for 2 hours), and dissolve in water to make exactly 200 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, Aₜ and Aₛ, of the sample solution and the standard solution at 280 nm as directed under Ultraviolet-visible Spectrophotometry <2,24>.

Dissolution rate (%) with respect to the labeled amount of methyldopa (C₁₀H₁₃NO₄)

\[
Mₛ = Mₛ \times Aₜ/ Aₛ \times V/ V \times 1/C \times 45
\]

Mₛ: Amount (mg) of methyldopa for assay, calculated on the dried basis

C: Labeled amount (mg) of methyldopa (C₁₀H₁₃NO₄) in 1 tablet

Assay Weigh accurately and powder not less than 20 Methyldopa Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of methyldopa (C₁₀H₁₃NO₄), add 50 mL of 0.05 mol/L sulfuric acid TS, shake thoroughly for 15 minutes, and add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter through a dry filter paper. Discard the first 20 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 0.11 g of Methyldopa RS (previously dried at 125°C for 2 hours, and determine the loss on drying <2,41>, dissolve in 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, add exactly 5 mL of iron (II) tartrate TS, and add ammonia-ammonium acetate buffer solution, pH 8.5, to make exactly 100 mL.

Containers and storage Containers—Well-closed containers.
**dl-Methylephedrine Hydrochloride**

**Identification** (1) Determine the absorption spectrum of a solution of dl-Methylephedrine Hydrochloride (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24> and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the infrared absorption spectrum of dl-Methylephedrine Hydrochloride, previously dried, as directed under Infrared Spectrophotometry <2.25> and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of dl-Methylephedrine Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

**pH** <2.54> The pH of a solution prepared by dissolving 1.0 g of dl-Methylephedrine Hydrochloride in 20 mL of water is between 4.5 and 6.0.

**Melting point** <2.60> 207 – 211°C

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of dl-Methylephedrine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of dl-Methylephedrine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 50 mg of dl-Methylephedrine Hydrochloride in 20 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peak of methylephedrine is not larger than the peak area of methylephedrine from the standard solution.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 257 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 13.6 g of potassium dihydrogen phosphate and 3 g of sodium 1-heptane sulfonate in 1000 mL of water, and adjust the pH to 2.5 with phosphoric acid. To 900 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of methylephedrine is about 10 minutes.

Time span of measurement: About 2 times as long as the retention time of methylephedrine beginning after the solvent peak.

**System suitability**—

Test for required detectability: To exactly 2 mL of the standard solution add water to make exactly 20 mL. Confirm that the peak area of methylephedrine obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the standard solution.

System performance: Dissolve 50 mg of dl-Methylephedrine Hydrochloride and 0.4 mg of methyl parahydroxybenzoate in 50 mL of water. When the procedure is run with 20 μL of this solution under the above operating conditions, methylephedrine and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methylephedrine is not more than 2.0%.

**Loss on drying** <2.47> Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of dl-Methylephedrine Hydrochloride, previously dried, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 21.57 mg of C₁₁H₁₇NO·HCl

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.
10% dl-Methylephedrine Hydrochloride Powder

dl-メチルエフェドリン塩酸塩散 10%

10% dl-Methylephedrine Hydrochloride Powder contains not less than 9.3% and not more than 10.7% of dl-methylephedrine hydrochloride (C₁₁H₁₇NO.HCl: 215.72).

Method of preparation

dl-Methylephedrine Hydrochloride 100 g
Starch, Lactose Hydrate or their mixture a sufficient quantity

To make 1000 g

Prepare as directed under Granules or Powders, with the above ingredients.

Identification Determine the absorption spectrum of a solution of 10% dl-Methylephedrine Hydrochloride Powder (1 in 200) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 250 nm and 253 nm, between 255 nm and 259 nm, and between 261 nm and 264 nm.

Assay Weigh accurately about 0.5 g of 10% dl-Methylephedrine Hydrochloride Powder, add exactly 4 mL of the internal standard solution and 25 mL of water, shake vigorously for 20 minutes to dissolve, add water to make 50 mL, filter through a membrane filter with pore size of 0.45 µm, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of dl-methylephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 4 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 µL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios of the peak area, Q₁ and Q₂, of methylephedrine to that of the internal standard.

Amount (mg) of dl-methylephedrine hydrochloride
(C₁₁H₁₇NO.HCl) = Mₛ x Q₁/Q₂

Mₛ: Amount (mg) of dl-methylephedrine hydrochloride for assay

Internal standard solution—A solution of methyl parahydroxybenzoate in acetonitrile (1 in 10,000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 257 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecysilanolized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 13.6 g of potassium dihydrogen phosphate and 3 g of sodium 1-heptane sulfonate in 1000 mL of water, and adjust the pH to 2.5 with phosphoric acid. To 900 mL of this solution add 200 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of methylephedrine is about 10 minutes.

System suitability—
System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, methylephedrine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.
System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methylephedrine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.

Methylergometrine Maleate
メチルエルゴメトリンマレイン酸塩

C₃₀H₄₂N₂O₆.C₄H₄O₄: 455.50
(8S)-N-[(1S)-1-(Hydroxymethyl)propyl]-6-methyl-9,10-didehydroergoline-8-carboxamide monomaleate [7054-07-1]

Methylergometrine Maleate, when dried, contains not less than 95.0% and not more than 105.0% of C₃₀H₄₂N₂O₆.C₄H₄O₄.

Description Methylergometrine Maleate occurs as a white to pale yellow, crystalline powder. It is odorless.
It is slightly soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether.
It gradually changes to yellow by light.
Melting point: about 190°C (with decomposition).

Identification (1) A solution of Methylergometrine Maleate (1 in 200) shows a blue fluorescence.
(2) The colored solution obtained in the Assay develops a deep blue in color. Determine the absorption spectrum of the colored solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methylergometrine Maleate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.
(3) To 5 mL of a solution of Methylergometrine Maleate (1 in 500) add 1 drop of potassium permanganate TS: the red color of the test solution fades immediately.

Optical rotation <2.49> [α]D Supplements: +44° to +50° (after drying,
Purity Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 8 mg of Methylergometrine Maleate in 2 mL of a mixture of ethanol (95) and ammonia solution (28) (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of ethanol (95) and ammonia solution (28) (9:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test immediately with these solutions as directed under Thin-layer Chromatography <2.67>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, and immediately develop the plate with a mixture of chloroform, methanol and water (75:25:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.47> Not more than 2.0% (0.2 g, in vacuum, phosphorus (V) oxide, 4 hours).

Assay Weigh accurately about 10 mg each of Methylergometrine Maleate and Methylergometrine Maleate RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution to a brown glass-stoppered centrifuge tube, add 30 mL of water, shake for 10 minutes vigorously, and centrifuge for 5 minutes. Discard the water layer, take the chloroform extracts, add chloroform to make exactly 10 mL of a solution containing about 5 μg of methylergometrine maleate (C₂₀H₂₅N₃O₂·C₄H₄O₄) per mL, and use this solution as the sample solution. Separately, weigh accurately about 1.25 mg of Methylergometrine Maleate RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution into a brown glass-stoppered centrifuge tube, add 3 g of sodium chloride and 2 mL of ammonia solution (28), shake vigorously for 10 minutes, centrifuge for 5 minutes. Discard the water layer, take the chloroform extracts, add chloroform to make exactly 10 mL of a solution containing about 5 μg of methylergometrine maleate (C₂₀H₂₅N₃O₂·C₄H₄O₄) per mL, and use this solution as the sample solution. Prepare as directed under Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, to exactly 10 mL of the subsequent filtrate add water to make exactly V mL so that each mL contains about 0.13 μg of methylergometrine maleate (C₂₀H₂₅N₃O₂·C₄H₄O₄) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Methylergometrine Maleate RS, previously dried in a desiccator for 4 hours (in vacuum, phosphorus (V) oxide), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, then pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine immediately the intensities of the subsequent solutions of the sample solution and standard solution at 545 nm, respectively.

Amount (mg) of C₂₀H₂₅N₃O₂·C₄H₄O₄

\[ M_s = \frac{M_x \times A_T}{A_S} \]

Where:

- \( M_s \): Amount (mg) of Methylergometrine Maleate RS
- \( M_x \): Amount (mg) of methylergometrine maleate (C₂₀H₂₅N₃O₂·C₄H₄O₄)
- \( A_T \): Absorbance of the sample solution
- \( A_S \): Absorbance of the standard solution
- \( V \): Volume in mL of the solution

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Methylergometrine Maleate Tablets

メチルエルゴメトリンメライン酸塩錠

Methylergometrine Maleate Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of methylergometrine maleate (C₂₀H₂₅N₃O₂·C₄H₄O₄: 455.50).

Method of preparation Prepare as directed under Tablets, with Methylergometrine maleate.

Identification (1) The sample solution obtained in the Assay shows a blue fluorescence. (2) The colored solution obtained in the Assay shows a deep blue color. Determine the absorption spectrum of the colored solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 543 nm and 547 nm and between 620 nm and 630 nm.

Uniformity of dosage unit <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.
fluorescence, \( F_t \) and \( F_s \), of the sample solution and standard solution at 338 nm as the excitation wavelength and at 427 nm as the fluorescence wavelength as directed under Fluorometry <2.22>.

Dissolution rate (%) with respect to the labeled amount of methylergometrine maleate (\( \text{C}_{20}\text{H}_{25}\text{N}_3\text{O}_2 \cdot \text{C}_4\text{H}_4\text{O}_4 \))

\[
M_s: \text{Amount (mg) of Methylergometrine Maleate RS}
\]

\[
\text{C}: \text{Labeled amount (mg) of methylergometrine maleate (C}_2\text{H}_3\text{N}_2\text{O}_2 \cdot \text{C}_4\text{H}_4\text{O}_4) \text{ in 1 tablet}
\]

\[
M_s = M_c / C 	imes V / V' 	imes 1 / C 	imes 9 / 20
\]

\( M_s \): Amount (mg) of Methylergometrine Maleate RS

\( C \): Labeled amount (mg) of methylergometrine maleate (\( \text{C}_2\text{H}_3\text{N}_2\text{O}_2 \cdot \text{C}_4\text{H}_4\text{O}_4 \)) in 1 tablet

**Assay**

Weigh accurately and powder not less than 20 Methylergometrine Maleate Tablets. Weigh accurately a portion of the powder, equivalent to about 0.3 mg of methylergometrine maleate (\( \text{C}_2\text{H}_3\text{N}_2\text{O}_2 \cdot \text{C}_4\text{H}_4\text{O}_4 \)), transfer to a brown separator, add 15 mL of sodium hydroxide carbonate solution (1 in 20), and extract with four 20-mL portions of chloroform. Filter each portion of the chloroform extracts through a pledget of absorbent cotton, previously moistened with chloroform, into another dried, brown separator, combine all the extracts, and use this extract as the standard solution. Separately, weigh accurately about 10 mg of Methylergometrine Maleate RS, previously dried in a desiccator (silica gel) for 4 hours, dissolve in water, and add water to make exactly 100 mL. Pipet 3 mL of this solution, and transfer to a brown separator, proceed in the same manner as the preparation of the sample solution, and use this extract as the standard solution. To each total volume of the sample solution and the standard solution add exactly 25 mL each of dilute \( \text{p}-\text{dimethylaminobenzaldehyde-ferric chloride TS} \), and after vigorous shaking for 5 minutes, allow to stand for 30 minutes. Draw off the water layer, centrifuge, and allow to stand for 1 hour. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using dilute \( \text{4-dimethylaminobenzaldehyde-ferric chloride TS} \) as the blank. Determine the absorbances, \( A_t \) and \( A_s \), of the subsequent solutions of the sample solution and the standard solution at 545 nm, respectively.

\[
\text{Amount (mg) of methylergometrine maleate (C}_2\text{H}_3\text{N}_2\text{O}_2 \cdot \text{C}_4\text{H}_4\text{O}_4) \text{ = M}_s \times V / V' 
\]

\[
\text{Amount (mg) of Methylergometrine Maleate RS = M}_s \times A_t / A_s \times 3 / 100
\]

Containers and storage

Containers—Well-closed containers.

Storage—Light-resistant.

**Methyl Parahydroxybenzoate**

パラオキシ安息香酸メチル

\( \text{C}_8\text{H}_8\text{O}_3: 152.15 \)

Methyl 4-hydroxybenzoate

\[ \text{C}_8\text{H}_8\text{O}_3 \text{ (98-76-3)} \]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (● ●).

Methyl Parahydroxybenzoate, when dried, contains not less than 98.0% and not more than 102.0% of \( \text{C}_8\text{H}_8\text{O}_3 \).

**Description**

Methyl Parahydroxybenzoate, occurs as colorless crystals or a white, crystalline powder.

It is freely soluble in ethanol (95) and in acetone, and slightly soluble in water.

**Identification**

(1) The melting point <2.60> of Methyl Parahydroxybenzoate is between 125°C and 128°C.

(2) Determine the infrared absorption spectrum of Methyl Parahydroxybenzoate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity**

(1) Clarity and color of solution—Dissolve 1.0 g of Methyl Parahydroxybenzoate in 10 mL of ethanol (95): the solution is clear and not more intensely colored than the following control solution.

Control solution: To 5.0 mL of Cobalt (II) Chloride CS, 12.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS add water to make 1000 mL.

(2) Acidity—Dissolve 0.20 g of Methyl Parahydroxybenzoate in 5 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mL of 0.1 mol/L sodium hydroxide VS: the solution shows a blue color.

(3) Heavy metals <1.07>—Dissolve 1.0 g of Methyl Parahydroxybenzoate in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 25 mL of acetone, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).

(4) Related substances—Dissolve 0.10 g of Methyl Parahydroxybenzoate in 10 mL of acetone, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 \( \mu \)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, water and acetic acid (100) (70:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot with the sample solution is not more intense than the spot obtained with the standard solution.

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay**

Weigh accurately about 1.0 g of Methyl Parahydroxybenzoate, add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate <2.50> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS = 152.1 mg of \( \text{C}_8\text{H}_8\text{O}_3 \).
Methylprednisolone

メチルプレドニゾロン

\[
\text{C}_{22}\text{H}_{30}\text{O}_5: 374.47
\]
11β,17,21-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione
[83-43-2]

Methylprednisolone, when dried, contains not less than 96.0% and not more than 104.0% of \( \text{C}_{22}\text{H}_{30}\text{O}_5 \).

**Description**
Methylprednisolone occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in methanol and in 1,4-dioxane, slightly soluble in ethanol (95) and in chloroform, and practically insoluble in water and in diethyl ether.

Melting point: 232 – 240°C (with decomposition).

**Identification**

1. Add 2 mL of sulfuric acid to 2 mg of Methylprednisolone: a deep red color develops with no fluorescence. Then add 10 mL of water to this solution: the color fades, and a gray, flocculent precipitate is produced.

2. Dissolve 10 mg of Methylprednisolone in 1 mL of methanol, add 1 mL of Fehling’s TS, and heat: a red precipitate is produced.

3. Determine the absorption spectrum of a solution of Methylprednisolone in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Optical rotation** <2.49> \([\alpha]_D^25 + 79 – +86^\circ\) (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

**Purity**
Related substances—Dissolve 50 mg of Methylprednisolone in 5 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (9:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, diethyl ether, methanol and water (385:75:40:6) to a distance of about 12 cm, and air-dry the plate. Then heat at 105°C for 10 minutes, cool, and spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 1.0% (0.5 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.2% (0.2 g).

**Assay**
Weigh accurately about 10 mg of Methylprednisolone, previously dried, and dissolve in methanol to make exactly 100 mL. To exactly 5 mL of this solution add methanol to make exactly 50 mL, and determine the absorbance \(A\) at the wavelength of maximum absorption at about 243 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of \( \text{C}_{22}\text{H}_{30}\text{O}_5 \) = \( A/400 \times 10,000 \)

**Containers and storage**
Containers—Well-closed containers.

Methylprednisolone Succinate

メチルプレドニゾロンコハク酸エステル

\[
\text{C}_{26}\text{H}_{34}\text{O}_8: 474.54
\]
11β,17,21-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione 21-(hydrogen succinate)
[2921-57-5]

Methylprednisolone Succinate, when dried, contains not less than 97.0% and not more than 103.0% of \( \text{C}_{26}\text{H}_{34}\text{O}_8 \).

**Description**
Methylprednisolone Succinate occurs as a white, crystals or crystalline powder.

It is soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in water.

Melting point: about 235°C (with decomposition).

**Identification**

1. Determine the absorption spectrum of a solution of Methylprednisolone Succinate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methylprednisolone Succinate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

2. Determine the infrared absorption spectrum of Methylprednisolone Succinate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Methylprednisolone Succinate RS: both spectra exhibit similar intensities of absorption at the same wave numbers. In case when some differences are found between the spectra, repeat the test with residues obtained by dissolving these substances in ethanol (95), evaporating to dryness, and drying.

**Optical rotation** <2.49> \([\alpha]_D^25 + 99 – +103^\circ\) (after drying, 0.2 g, ethanol (95), 20 mL, 100 mm).

**Purity**
Heavy metals <1.07>—Proceed with 1.0 g of Methylprednisolone Succinate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of
Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.1>—Prepare the test solution with 2.0 g of Methylprednisolone Succinate according to Method 3, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 15 mg of Methylprednisolone Succinate in 5 mL of methanol, add a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (1:1) to make exactly 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add exactly 5 mL of the mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the areas of peaks of methylprednisolone succinate from the standard solution, and the total area of the peaks other than the peak of methylprednisolone succinate from the standard solution, is not larger than 1/2 times the peak area of methylprednisolone succinate from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of methylprednisolone succinate.

**System suitability**

Test for required detectability: Pipet 1 mL of the standard solution, and add the mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (1:1) to make exactly 10 mL. Confirm that the peak area of methylprednisolone succinate obtained from 5 µL of this solution is equivalent to 7 to 13% of that from 5 µL of the standard solution.

System performance: Proceed as directed in the System suitability in the Assay.

System repeatability: When the test is repeated 6 times with 5 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methylprednisolone succinate is not more than 1.0%.

**Loss on drying** <2.4> Not more than 1.0% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.2% (0.5 g).

**Assay** Weigh accurately about 15 mg each of Methylprednisolone Succinate and Methylprednisolone Succinate RS, previously dried, dissolve separately in 5 mL of methanol, and add the mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (1:1) to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q1 and Q2, of the peak area of methylprednisolone succinate to that of the internal standard.

Amount (mg) of \( M \) = \( M_s \times Q/Q_s \)

\( M_s \) = Amount (mg) of Methylprednisolone Succinate RS

**Internal standard solution**—A solution of ethyl parahydroxybenzoate in a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (1:1) (3 in 20,000).

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 1000 mL of 0.05 mol/L potassium dihydrogen phosphate TS add a suitable amount of 0.05 mol/L disodium hydrogen phosphate TS to make a solution having pH 5.5. To 640 mL of this solution add 360 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of methylprednisolone succinate is about 6 minutes.

**System suitability**

System performance: When the procedure is run with 5 µL of the standard solution under the above operating conditions, methylprednisolone succinate and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 5 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methylprednisolone succinate to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

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**Methylrosanilinium Chloride**

**Crystal Violet**

メチルロザニリン塩化物

\( C_{25}H_{30}ClN_3: 407.98 \)

Methylrosanilinium Chloride is hexamethylpararosaniline chloride, and is usually admixed with pentamethylpararosaniline chloride and tetramethylpararosaniline chloride.

It contains not less than 96.0% of methylrosanilinium chloride [as hexamethylpararosaniline chloride (\( C_{25}H_{30}ClN_3 \))], calculated on the dried basis.

**Description** Methylrosanilinium Chloride occurs as green fragments having a metallic luster or a dark green powder. It is odorless or has a slight odor.

It is soluble in ethanol (95), sparingly soluble in water, and practically insoluble in diethyl ether.

**Identification**

(1) To 1 mL of sulfuric acid add 1 mg of Methylrosanilinium Chloride: it dissolves, and shows an orange to red-brown color. To this solution add water dropwise: the color of the solution changes from brown through green to blue.

(2) Dissolve 0.02 g of Methylrosanilinium Chloride in 10 mL of water, add 5 drops of hydrochloric acid, and use this solution as the sample solution. To 5 mL of the sample solution add tannic acid TS dropwise: an intense blue precipitate is formed.

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Methyl Salicylate

**C₈H₈O₃**: 152.15

Methyl 2-hydroxybenzoate [119-36-8]

Methyl Salicylate contains not less than 98.0% of C₈H₈O₃.

**Description**
Methyl Salicylate is a colorless to pale yellow liquid. It has a strong, characteristic odor. It is miscible with ethanol (95) and with diethyl ether. It is very slightly soluble in water.

**Specific gravity**

\[ d_{20}^2: 1.182 – 1.192 \]

**Boiling point**: 219 – 224°C

**Identification**
Shake 1 drop of Methyl Salicylate thoroughly with 5 mL of water for 1 minute, and add 1 drop of iron (III) chloride TS; a purple color develops.

**Purity (1)**

**Acidity**—Shake 5.0 mL of Methyl Salicylate thoroughly with 25 mL of freshly boiled and cooled water and 1.0 mL of 0.1 mol/L sodium hydroxide VS for 1 minute, add 2 drops of phenol red TS, and titrate with 0.1 mol/L hydrochloric acid VS until the red color disappears: not more than 0.45 mL of 0.1 mol/L sodium hydroxide VS is consumed.

**Heavy metals**—Shake 10.0 mL of Methyl Salicylate thoroughly with 10 mL of water, add 1 drop of hydrochloric acid, and saturate with hydrogen sulfide by passing it through the mixture: neither the oily layer nor the aqueous layer shows a dark color.

**Assay**
Weigh accurately about 2 g of Methyl Salicylate, add an exactly measured 50 mL of 0.5 mol/L potassium hydroxide-ethanol VS, and heat on a water bath for 2 hours under a reflux condenser. Cool, and titrate with 0.5 mol/L potassium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

\[ \text{Each mL of 0.5 mol/L potassium hydroxide-ethanol VS} = 76.08 \text{ mg of C}_8\text{H}_8\text{O}_3 \]

Containers and storage—Tight containers.

**Containers and storage**

Containers—Tight containers.
Compound Methyl Salicylate Spirit

複方サリチル酸メチル精

Method of preparation

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl Salicylate</td>
<td>40 mL</td>
</tr>
<tr>
<td>Capsicum Tincture</td>
<td>100 mL</td>
</tr>
<tr>
<td>d- or dl-Camphor</td>
<td>50 g</td>
</tr>
<tr>
<td>Ethanol</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Prepare as directed under Medicated Spirits, with the above ingredients.

Description Compound Methyl Salicylate Spirit is a reddish yellow liquid, having a characteristic odor and a burning taste.

Identification (1) Shake 1 mL of Compound Methyl Salicylate Spirit with 5 mL of dilute ethanol, and add 1 drop of iron (III) chloride TS: a purple color is produced (methyl salicylate).

(2) Shake thoroughly 0.5 mL of Compound Methyl Salicylate Spirit with 10 mL of chloroform, and use this solution as the sample solution. Perform the test with these solutions as the sample solution and standard solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and chloroform (4:1) to a distance of about 10 cm, air-dry the plate, and examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot upon the plate: the spot from the standard solution and the corresponding spot from the sample solution reveal a purple color.

Containers and storage Containers—Tight containers.

Methyltestosterone

メチルテストレロン

C_{20}H_{30}O_2: 302.45
17β-Hydroxy-17α-methylandrost-4-en-3-one
[38-18-4]

Methyltestosterone, when dried, contains not less than 98.0% and not more than 102.0% of C_{20}H_{30}O_2.

Description Methyltestosterone occurs as white to pale yellow, crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Methyltestosterone in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methyltestosterone RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Methyltestosterone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Methyltestosterone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]_D^{20}: +79 – +85° (after drying, 0.1 g, ethanol (95), 10 mL, 100 mm).

Melting point <2.60> 163 – 168°C

Purity Related substances—Dissolve 40 mg of Methyltestosterone in 2 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and diethylamine (19:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (0.5 g, in vacuum, phosphorus (V) oxide, 10 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 20 mg each of Methyltestosterone and Methyltestosterone RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 10 hours, dissolve each in methanol to make exactly 200 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of methyltestosterone to that of the internal standard.

\[ \text{Amount (mg) of } C_{20}H_{30}O_2 = M_S \times Q_T/Q_S \]

\[ M_S: \text{Amount (mg) of Methyltestosterone RS} \]

Internal standard solution—A solution of propyl parahydroxybenzoate in methanol (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 241 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about
Methylenetetrahydrofuran Tablets 肝移植用メチルテストステロン錠

Methylenetetrahydrofuran Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of methylenetetrahydrofuran (C20H30O2: 302.45).

Method of preparation Prepare as directed under Tablets, with Methylenetetrahydrofuran.

Identification To a portion of powdered Methylenetetrahydrofuran Tablets, equivalent to 10 mg of Methylenetetrahydrofuran according to the labeled amount, add 50 mL of acetone, shake for 30 minutes, and filter. Evaporate the filtrate to dryness, dissolve the residue in 10 mL of acetone, and use this solution as the sample solution. Separately, dissolve 10 mg of Methylenetetrahydrofuran RS in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95:9:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 110°C for 10 minutes: the spot from the sample solution and the standard solution show the same Rf value.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Methylenetetrahydrofuran Tablets add 5 mL of water to disintegrate, add 50 mL of methanol, and shake for 30 minutes. Add methanol to make exactly 100 mL, and centrifuge. Measure exactly V mL of the supernatant liquid, add methanol to make exactly V' mL of a solution containing about 10 μg of methylenetetrahydrofuran (C20H30O2) per mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Methylenetetrahydrofuran RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 10 hours, and dissolve in 5 mL of water and 50 mL of methanol, then add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A1 and A5, of the sample solution and the standard solution at the wavelength of maximum absorption at about 241 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry <2.25>.

\[
\text{Amount (mg) of methylenetetrahydrofuran (C}_{20}\text{H}_{30}\text{O}_{2}) = M_5 \times A_1/A_S \times V'/V \times 1/10
\]

M5: Amount (mg) of Methylenetetrahydrofuran RS

Dissolution <6.10> When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of a solution prepared by dissolving 1 g of polysorbate 80 in water to make 5 L as the dissolution medium, the dissolution rate in 30 minutes of a 10-mg tablet is not less than 75% and that in 60 minutes of a 25-mg tablet is not less than 70%.

Start the test with 1 tablet of Methylenetetrahydrofuran Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V mL so that each mL contains about 11 μg of methylenetetrahydrofuran (C20H30O2) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Methylenetetrahydrofuran RS, previously dried in vacuum using phosphorus (V) oxide as a desiccant for 10 hours, and dissolve in ethanol (99.5) to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A1 and A5, at 249 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of methylenetetrahydrofuran (C20H30O2)

\[
\text{Dissolution rate} = \left(1 - \frac{A_1}{A_5}\right) \times \frac{100}{C} \times \frac{1/45}{M_5}
\]

M5: Amount (mg) of Methylenetetrahydrofuran RS
C: Labeled amount (mg) of methylenetetrahydrofuran (C20H30O2) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Methylenetetrahydrofuran Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg of methylenetetrahydrofuran (C20H30O2), add about 70 mL of methanol, shake for 30 minutes, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution and methanol to make 50 mL, filter through a membrane filter (not exceeding 0.45 μm in pore size), and use the filtrate as the sample solution. Separately, weigh accurately about 20 mg of Methylenetetrahydrofuran RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 10 hours, dissolve in methanol to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q1 and Q5, of the peak area of methylenetetrahydrofuran to that of the internal standard.
Amount (mg) of methyltestosterone \((C_{20}H_{30}O_{2})\)
\[
M_s = M_t \times \frac{Q_t}{Q_s} \times \frac{5}{4}
\]

\(M_t\): Amount (mg) of Methyltestosterone RS

Internal standard solution—A solution of propyl parahydroxybenzoate in methanol (1 in 10,000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 241 nm).
Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).
Column temperature: A constant temperature of about 35°C.
Mobile phase: A mixture of acetonitrile and water (11:9).
Flow rate: Adjust the flow rate so that the retention time of methyltestosterone is about 10 minutes.

System suitability—
System performance: When the procedure is run with 10 \(\mu\)L of the standard solution under the above operating conditions, the internal standard and methyltestosterone are eluted in this order with the resolution between these peaks being not less than 9.
System repeatability: When the test is repeated 6 times with 10 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methyltestosterone to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Tight containers.

Meticrane

メチクラン

\(\text{C}_{10}\text{H}_{13}\text{NO}_{4}\text{S}_{2}\) : 275.34
6-Methylthiochromane-7-sulfonamide 1,1-dioxide

Meticrane, when dried, contains not less than 98.0% of \(\text{C}_{10}\text{H}_{13}\text{NO}_{4}\text{S}_{2}\).

Description—Meticrane occurs as white, crystals or crystalline powder. It is odorless and has a slight bitter taste.

It is freely soluble in dimethylformamide, slightly soluble in acetonitrile and in methanol, very slightly soluble in ethanol (95), and practically insoluble in water.

Melting point: about 234°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Meticrane in methanol (3 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Meticrane, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Ammonium <1.02>—Perform the test with 0.10 g of Meticrane. Prepare the control solution with 3.0 mL of Standard Ammonium Solution (not more than 0.03%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Meticrane according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.17>—Prepare the test solution with 1.0 g of Meticrane according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Meticrane in 50 mL of acetonitrile. To 5 mL of this solution add the mobile phase to make 25 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 \(\mu\)L of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than meticrane from the sample solution is not larger than the peak area of meticrane from the standard solution.

Operating conditions 1—
Detector: An ultraviolet absorption photometer (wavelength: 230 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of water and acetonitrile (17:3).
Flow rate: Adjust the flow rate so that the retention time of meticrane is about 7 minutes.

Time span of measurement: About 4 times as long as the retention time of meticrane beginning after the solvent peak.

System suitability 1—
Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of meticrane obtained from 10 \(\mu\)L of this solution is equivalent to 7 to 13% of that from 10 \(\mu\)L of the standard solution.

System performance: Dissolve 10 mg each of Meticrane and caffeine in 100 mL of acetonitrile. To exactly 2 mL of this solution add the mobile phase to make exactly 10 mL. When the procedure is run with 10 \(\mu\)L of this solution under the above operating conditions 1, caffeine and meticrane are eluted in this order with the resolution between these peaks being not less than 10.
System repeatability: When the test is repeated 6 times with 10 \(\mu\)L of the standard solution under the above operating conditions 1, the relative standard deviation of the peak area of meticrane is not more than 2.0%.

Operating conditions 2—
Detector, column, and column temperature: Proceed as directed in the operating conditions 1.
Mobile phase: A mixture of water and acetonitrile (1:1).
Flow rate: Adjust the flow rate so that the retention time of meticrane is about 2 minutes.
Time span of measurement: About 10 times as long as the retention time of meticrane beginning after the solvent peak.

System suitability 2—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of meticrane obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: Dissolve 20 mg each of Meticrane and methyl parahydroxybenzoate in 100 mL of acetonitrile. To exactly 2 mL of this solution add the mobile phase to make exactly 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions 2, meticrane and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions 2, the relative standard deviation of the peak area of meticrane is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Meticrane, previously dried, dissolve in 50 mL of dimethylformamide, add 5 mL of water, and titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 27.54 mg of C_{10}H_{13}NO_{4}S_{2}

Containers and storage Containers—Well-closed containers.

Metildigoxin
メチルジゴキシン

C_{42}H_{66}O_{14}·\frac{1}{2}C_{3}H_{6}O: 824.00
3\beta-[2,6-Dideoxy-4-O-methyl-β-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-ribo-hexopyranosyl oxy]-12β,14-dihydroxy-5β-card-20(22)-enolide—acetone (1/1) [30683-43-9, acetone-free]

Metildigoxin contains not less than 96.0% and not more than 103.0% of C_{42}H_{66}O_{14}·\frac{1}{2}C_{3}H_{6}O, calculated on the anhydrous basis.

Description Metildigoxin occurs as a white to light yellowish white, crystalline powder.

It is freely soluble in N,N-dimethylformamide, in pyridine and in acetic acid (100), soluble in chloroform, sparingly soluble in methanol, slightly soluble in ethanol (95) and in acetone, very slightly soluble in water, and practically insoluble in diethyl ether.

Identification (1) Dissolve 2 mg of Metildigoxin in 2 mL of acetic acid (100), shake well with 1 drop of iron (III) chloride TS, and add gently 2 mL of sulfuric acid to divide into two layers: a brown color develops at the interface, and a deep blue color gradually develops in the acetic acid layer.

(2) Dissolve 2 mg of Metildigoxin in 2 mL of 1,3-dinitrobenzene TS, add 2 mL of a solution of tetramethylammonium hydroxide in ethanol (95) (1 in 200), and shake: a purple color gradually develops, and changes to blue-purple.

(3) Determine the absorption spectrum of a solution of Metildigoxin in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Metildigoxin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Metildigoxin and Metildigoxin RS in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

(4) Determine the infrared absorption spectrum of Metildigoxin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Metildigoxin RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Metildigoxin and Metildigoxin RS in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.
Optical rotation $\theta_{2.49}$ [α]$_{D}^{20}$: $+22.0$– $+25.5^\circ$ (1 g, calculated on the anhydrous basis, pyridine, 10 mL, 100 mm).

**Purity (1)** Arsenic $<1.17$—Prepare the test solution with 0.5 g of Metildigoxin according to Method 3, and perform the test (not more than 4 ppm).

(2) Related substances—Dissolve 10 mg of Metildigoxin in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.02>$. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-butanone and chloroform (3:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 110°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Acetone** Weigh accurately about 0.1 g of Metildigoxin, dissolve in exactly 2 mL of the internal standard solution, add $N,N$-dimethylformamide to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of acetone in a 50-mL volumetric flask containing about 10 mL of $N,N$-dimethylformamide, and add $N,N$-dimethylformamide to make 50 mL. Pipet 5 mL of this solution, add exactly 20 mL of the internal standard solution, then add $N,N$-dimethylformamide to make 100 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography $<2.02>$. Calculate the ratios, $Q_t$ and $Q_s$, of the peak area of acetone to that of the internal standard: the amount of acetone is between 2.0% and 5.0%.

Amount (%) of acetone $= M_s/M_t \times Q_t/Q_s$

$M_s$: Amount (g) of acetone
$M_t$: amount (g) of the sample

**Internal standard solution**—A solution of $t$-butanol in $N,N$-dimethylformamide (1 in 2000).

**Operating conditions**—
Detector: A hydrogen flame-ionization detector.
Column: A glass column about 2 mm in inside diameter and 1 to 2 m in length, packed with porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (150 to 180 μm in particle diameter).
Column temperature: A constant temperature between 170°C and 230°C.
Carrier gas: Nitrogen.
Flow rate: Adjust the flow rate so that the retention time of acetone is about 2 minutes.
Selection of column: Proceed with 1 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of acetone and $t$-butanol in this order with the resolution between these peaks being not less than 2.0.

**Water $<2.48>$** Not more than 3.0% (0.3 g, volumetric titration, direct titration).

**Residue on ignition $<2.48>$** Not more than 0.1% (0.5 g).

**Assay** Weigh accurately 0.1 g each of Metildigoxin and Metildigoxin RS (separately, determine the water $<2.48>$ in the same manner as Metildigoxin), and dissolve each in methanol to make exactly 50 mL. Pipet 5 mL each of the solutions, add methanol to each to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Pipet 5 mL each of the sample solution and standard solution, add 15 mL of 2,4,6-trinitrophenol-ethanol TS and 2 mL of sodium hydroxide TS to each, shake well, add methanol to make exactly 25 mL, and allow to stand at 20 ± 0.5°C for 20 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry $<2.24>$ using a solution prepared by mixing 15 mL of 2,4,6-trinitrophenol-ethanol TS and 2 mL of sodium hydroxide TS and adding methanol to make exactly 25 mL as the blank. Determine the maximum absorbances, $A_t$ and $A_s$, of the subsequent solutions obtained from the sample solution and the standard solution, respectively, by measuring every 5 minutes, at 495 nm.

Amount (mg) of $C_2H_5OH$: $\frac{1}{7}C_3H_6O$

$M_s$: Amount (mg) of Metildigoxin RS, calculated on the anhydrous basis.

**Containers and storage** Containers—Tight containers.

### Metoclopramide

メトクロプラミド

![Metoclopramide structure](https://example.com/metoclopramide_structure)

$C_{14}H_{22}ClN_3O_2$: 299.80

4-Amino-5-chloro-N-[2-(diethylamino)ethyl]-2-methoxybenzamide

[364-62-5]

Metoclopramide, when dried, contains not less than 99.0% of $C_{14}H_{22}ClN_3O_2$.

**Description** Metoclopramide occurs as white crystals or a crystalline powder, and is odorless. It is freely soluble in acetic acid (100), soluble in methanol and in chloroform, sparingly soluble in acetic anhydride, in ethanol (95) and in acetone, very slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

**Identification (1)** Dissolve 10 mg of Metoclopramide in 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests $<1.09>$ for Primary Aromatic Amines.

(2) Dissolve 10 mg of Metoclopramide in 5 mL of dilute hydrochloric acid and 20 mL of water, and to 5 mL of this solution add 1 mL of Dragendorff’s TS: a reddish orange precipitate is produced.

(3) Dissolve 0.1 g of Metoclopramide in 1 mL of 1 mol/L hydrochloric acid TS, and dilute with water to make 100 mL. To 1 mL of the solution add water to make 100 mL,
determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <2.60> 146 – 149°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Metoclopramide in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Metoclopramide as directed under Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Dissolve 1.0 g of Metoclopramide in 5 mL of 1 mol/L hydrochloric acid TS, and use this solution as the sample solution. Perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Metoclopramide in 10 mL of methanol, and use this solution as the sample solution. Dilute 1 mL of the sample solution, exactly measured, with methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol and ammonia solution (28:19:1) to a distance of about 10 cm. Dry the plate, first in air and then at 80°C for 30 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Dissolve about 0.4 g of Metoclopramide, previously dried and accurately weighed, in 50 mL of acetic acid (100), add 5 mL of acetic anhydride, and warm for 5 minutes. Allow to cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform the blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 29.98 mg of C14H22ClN3O2

Containers and storage Containers—Well-closed containers.

Metoclopramide Tablets

メトクロプラミド錠

Metoclopramide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of metoclopramide (C14H22ClN3O2: 299.80).

Method of preparation Prepare as directed under Tablets, with Metoclopramide.

Identification (1) To a quantity of powdered Metoclopramide Tablets, equivalent to 50 mg of Metoclopramide according to the labeled amount, add 15 mL of 0.5 mol/L hydrochloric acid TS, and heat in a water bath at 70°C for 15 minutes while frequent shaking. After cooling, centrifuge for 10 minutes, and to 5 mL of the supernatant liquid add 1 mL of 4-dimethylaminobenzaldehyde-hydrochloric acid TS: a yellow color develops.

(2) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 270 nm and 274 nm, and between 306 nm and 310 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Metoclopramide Tablets add 10 mL of 0.1 mol/L hydrochloric acid TS, disperse the particles with the aid of ultrasonic waves, then add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and centrifuge for 10 minutes. Pipet 4 mL of the supernatant liquid, add 0.1 mol/L hydrochloric acid TS to make exactly 5 mL of a solution so that each mL contains about 12 μg of metoclopramide (C14H22ClN3O2), and use this solution as the sample solution. Separately, weigh accurately about 80 mg of metoclopramide for assay, previously dried at 105°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Pipet 4 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A1 and A2, of the sample solution and standard solution at 308 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of metoclopramide (C14H22ClN3O2) = M × A1/A2 × V/1000

M3: Amount (mg) of metoclopramide for assay

Dissolution Being specified separately.

Assay Weigh accurately not less than 20 Metoclopramide Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 75 mg of metoclopramide (C14H22ClN3O2), add 300 mL of 0.1 mol/L hydrochloric acid TS, shake for 1 hour, and add 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Centrifuge for 10 minutes, pipet 4 mL of the supernatant liquid, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of metoclopramide for assay, previously dried at 105°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Pipet 4 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A1 and A2, of the sample solution and standard solution at 308 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of metoclopramide (C14H22ClN3O2) = M × A1/A2

M3: Amount (mg) of metoclopramide for assay

Containers and storage Containers—Tight containers.
Metoprolol Tartrate

メトプロロール酒石酸塩

\[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6 : 684.81\]

(2R,5)-1-[4-(2-Methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol hemi-(2R,3R)-tartrate [56392-17-7]

Metoprolol Tartrate, when dried, contains not less than 99.0% and not more than 101.0% of \((C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6\).

**Description** Metoprolol Tartrate occurs as a white crystalline powder.

It is very soluble in water, and freely soluble in methanol, in ethanol (95) and in acetic acid (100).

Optical rotation \([\alpha]_D^0 = +7.0 - +10.0^\circ\) (after drying, 1 g, water, 50 mL, 100 mm).

**Identification** (1) Determine the absorption spectrum of a solution of Metoprolol Tartrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Metoprolol Tartrate, previously dried, as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize Metoprolol Tartrate from a solution in acetone (23 in 10000), filter and dry the crystals, and perform the test with the crystals.

(3) A solution of Metoprolol Tartrate (1 in 5) responds to the Qualitative Tests <1.09> (1) for tartrate.

**pH** <2.54> The pH of a solution obtained by dissolving 1.0 g of Metoprolol Tartrate in 10 mL of water is between 6.0 and 7.0.

**Purity** (1) Heavy metals <1.07>—Proceed with 2.0 g of Metoprolol Tartrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.10 g of Metoprolol Tartrate in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. After saturating the plate with the atmosphere by allowing to stand in a developing vessel, which contains the developing solvent and a glass vessel containing ammonia water (28), develop with the developing solvent, a mixture of ethyl acetate and methanol (4:1), to a distance of about 12 cm, and air-dry the plate. Allow to stand the plate in an iodine vapors until the spot with the standard solution appears obviously: the spot other than the principal spot and other than the spot on the original point with the sample solution is not more than three spots, and they are not more than intense than the spot with the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (1 g, in vacuum, 60°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Metoprolol Tartrate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.26> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS is equivalent to 34.24 mg of \((C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6\).

**Containers and storage** Containers—Well-closed containers.

Metoprolol Tartrate Tablets

メトプロロール酒石酸塩錠

Metoprolol Tartrate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of metoprolol tartrate \((C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6 : 684.81\).

**Method of preparation** Prepare as directed under Tablets, with Metoprolol Tartrate.

**Identification** To an amount of powdered Metoprolol Tartrate Tablets, equivalent to 10 mg of Metoprolol Tartrate according to the labeled amount, add 100 mL of ethanol (95), shake for 15 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 274 nm and 278 nm and between 281 nm and 285 nm.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Metoprolol Tartrate Tablets add 1 mL of water for every 10 mg of Metoprolol Tartrate, shake for 20 minutes, then add 75 mL of ethanol (95), shake for 15 minutes, add ethanol (95) to make exactly 100 mL, and centrifuge. Pipet \(V\) mL of the supernatant liquid, add ethanol (95) to make exactly \(V\) so that each mL contains about 0.1 mg of metoprolol tartrate \(((C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6)\), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of metoprolol tartrate (as the sample solution). So that each mL contains about 0.1 mg of metoprolol tartrate \(((C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6)\), and use this solution as the standard solution. Separately, weigh accurately about 50 mg of metoprolol tartrate (as the sample solution). Determine the absorbances, \(A_T\) and \(A_S\), of the sample solution and standard solution at 276 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using ethanol (95) as the blank.
Amount (mg) of metoprolol tartrate \((\text{C}_{15}\text{H}_{25}\text{NO}_3)_2\cdot\text{C}_4\text{H}_6\text{O}_{6})\)

\[ M_S = M_s \times \frac{A_1}{A_3} \times \frac{V'}{V} \times 1/5 \]

Dissolution

When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Metoprolol Tartrate Tablets is not less than 80%.

Start the test with 1 tablet of Metoprolol Tartrate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add water to make exactly 100 mL so that each mL contains about 22 μg of metoprolol tartrate \((\text{C}_{15}\text{H}_{25}\text{NO}_3)_2\cdot\text{C}_4\text{H}_6\text{O}_{6})\) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 56 mg of metoprolol tartrate for assay, previously dried in vacuum at 60°C for 4 hours, dissolve in 60 mL of the mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1), add exactly 10 mL of the internal standard solution, then add the mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1) to make 100 mL, and use this solution as the standard solution.

Perform the test with exactly 50 mL of the internal standard solution, and use this solution as the standard solution as directed under Liquid Chromatography.<2.01> according to the following conditions, and calculate the ratios, \(Q_T\) and \(Q_S\), of the peak area of metoprolol to that of the internal standard.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter). Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 14.0 g of sodium perchlorate monohydrate in 1000 mL of water, and adjust to pH 3.2 with diluted perchloric acid (17 in 2000). To 750 mL of this solution add 250 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of metoprolol is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, metoprolol and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of metoprolol to that of the internal standard is not more than 1.0%.

Containers and storage—

Containers—Well-closed containers.

Metronidazole

メトロニダゾール

\(\text{C}_9\text{H}_8\text{N}_2\text{O}_3: 171.15\)

2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethanol [445-48-1]

Metronidazole, when dried, contains not less than 99.0% and not more than 101.0% of \(\text{C}_9\text{H}_8\text{N}_2\text{O}_3\).

Description

Metronidazole occurs as white to pale yellowish white crystals or crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in ethanol (99.5) and in acetone, and slightly soluble in water. It dissolves in dilute hydrochloric acid.

It is colored to yellow-brown by light.

Identification (1) Determine the absorption spectrum of a
solution of Metronidazole in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Metronidazole as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 159 – 163°C.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Metronidazole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) 2-Methyl-5-nitroimidazol—Dissolve 0.10 g of Metronidazole in acetone to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 20 mg of 2-methyl-5-nitroimidazole for thin-layer chromatography in acetone to make exactly 20 mL, then pipet 5 mL of this solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>.

Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Immediately develop the plate with a mixture of acetone, water and ethyl acetate (8:1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution corresponding to the spot from the standard solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 24 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Metronidazole, previously dried, and dissolve in 30 mL of acetic acid (100). Titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 0.5 mL of p-naphtholbenzoin TS) until the color of the solution changes from orange-yellow to green. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 17.12 mg of C6H9N3O3

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Metronidazole Tablets

メトロニダゾール錠

Metronidazole Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of metronidazole (C6H9N3O3; 171.15).

Method of preparation Prepare as directed under Tablets, with Metronidazole.

Identification (1) To an amount of powdered Metronida-
Dissolution rate (％) with respect to the labeled amount of metronidazole (C₆H₉N₃O₃)

\[ M_S \times \frac{A_T}{A_S} \times V/V \times 1/C \times 45 \]

\[ M_S \text{: Amount (mg) of metronidazole for assay} \]

\[ C \text{: Labeled amount (mg) of metronidazole (C₆H₉N₃O₃) in 1 tablet} \]

**Assay**

Weigh accurately the mass of not less than 20 Metronidazole Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.25 g of metronidazole (C₆H₉N₃O₃), add 25 mL of a mixture of water and methanol (1:1), shake vigorously for 10 minutes, and add the mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 5 mL of this solution, and add a mixture of water and methanol (4:1) to make exactly 100 mL. Filter this solution through a membrane filter with pore size of 0.45 μm, discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of metronidazole for assay, previously dried in vacuum on silica gel for 24 hours, dissolve in the mixture of water and methanol (4:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography for the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of metronidazole.

\[ \text{Amount (mg) of metronidazole (C₆H₉N₃O₃)} \]

\[ = M_S \times \frac{A_T}{A_S} \times 10 \]

\[ M_S \text{: Amount (mg) of metronidazole for assay} \]

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 320 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (4:1).

Flow rate: Adjust the flow rate so that the retention time of metronidazole is about 5 minutes.

**System suitability—**

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of metronidazole are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of metronidazole is not more than 1.0%.

**Containers and storage**

Containers—Tight containers.

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**Metyrapone**

メチラポン

C₁₄H₁₄N₂O: 226.27

2-Methyl-1,2-di(pyridin-3-yl)propan-1-one

[34-36-4]

Metyrapone, when dried, contains not less than 98.0% of C₁₄H₁₄N₂O.

**Description**

Metyrapone occurs as a white to pale yellow, crystalline powder. It has a characteristic odor and a bitter taste.

It is very soluble in methanol, in ethanol (95), in acetic anhydride, in chloroform, in diethyl ether and in nitrobenzene, and sparingly soluble in water.

It dissolves in 0.5 mol/L sulfuric acid TS.

**Identification**

1. Mix 5 mg of Metyrapone with 10 mg of 1-chloro-2,4-dinitrobenzene, melt by gently heating for 5 to 6 seconds, cool, and add 4 mL of potassium hydroxide-ethanol TS: a dark red color develops.

2. Determine the absorption spectrum of a solution of Metyrapone in 0.5 mol/L sulfuric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry for the following conditions, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Melting point**

\(<2.60\> 50 - 54°C.

**Purity**

Clarity and color of solution—Dissolve 0.5 g of Metyrapone in 5 mL of methanol: the solution is clear and colorless to pale yellow.

Heavy metals—Proceed with 2.0 g of Metyrapone according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Arsenic—Prepare the test solution with 1.0 g of Metyrapone, according to Method 3, and perform the test (not more than 2 ppm).

Related substances—Dissolve 0.25 g of Metyrapone in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 2 μL of each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (15:1) to a distance of about 10 cm, and air-dry the plate for about 15 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

**Loss on drying**

\(<2.4\> Not more than 0.5% (1 g, in vacuum, silica gel, 24 hours).
Residue on ignition \(<2.44\) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.2 g of Metyrapone, previously dried, dissolve in 10 mL of nitrobenzene and 40 mL of acetic anhydride, and titrate \(<2.50\) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 11.31 mg of \(\text{C}_4\text{H}_8\text{N}_2\text{O}\)

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

### Mexiletine Hydrochloride

メキシレチン塩酸塩

\[
\text{C}_{11}\text{H}_{17}\text{NO}.\text{HCl}: 215.72
\]

(1RS)-2-(2,6-Dimethylphenoxy)-1-methylethylamine monohydrochloride

**Description** Mexiletine Hydrochloride occurs as a white powder.

It is freely soluble in water and in ethanol (95), slightly soluble in acetonitrile, and practically insoluble in diethyl ether.

A solution of Mexiletine Hydrochloride (1 in 20) shows no optical rotation.

**Identification** (1) Determine the absorption spectrum of a solution of Mexiletine Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry \(<2.27\), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mexiletine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mexiletine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.29\), and compare the spectrum with the Reference Spectrum or the spectrum of dried Mexiletine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize Mexiletine Hydrochloride from ethanol (95), filter, dry the crystals, and repeat the test on the crystals.

(3) A solution of Mexiletine Hydrochloride (1 in 100) responds to the Qualitative Tests \(<1.09\) (2) for chloride.

**pH** \(<2.54\) Dissolve 1.0 g of Mexiletine Hydrochloride in 10 mL of water: the pH of this solution is between 3.8 and 5.8.

**Melting point** \(<2.60\) 200 – 204°C.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Mexiletine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy Metals \(<1.07\)—Proceed with 2.0 g of Mexiletine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 20 mg of Mexiletine Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 250 mL, and use this solution as the standard solution. Perform the test with exactly 20 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.07\) according to the following conditions. Determine each peak area of both solutions by the automatic integration method: each peak area of the peaks other than the peak of mexiletine from the sample solution is not larger than the peak area of mexiletine from the standard solution.

**Operating conditions**—

Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed as directed in the operating conditions in the Assay.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of mexiletine obtained from 20 \(\mu\)L of the standard solution is between 5 mm and 10 mm.

Time span of measurement: About 3 times as long as the retention time of mexiletine beginning after peaks of the solvent.

**Loss on drying** \(<2.41\) Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition \(<2.44\) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 20 mg each of Mexiletine Hydrochloride and Mexiletine Hydrochloride RS, each previously dried, and dissolve each in the mobile phase to make exactly 20 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.07\) according to the following conditions, and calculate the ratios, \(Q_1\) and \(Q_2\), of the peak area of mexiletine to that of the internal standard, respectively.

\[
\text{Amount (mg)} = M_5 \times \frac{Q_1}{Q_2}
\]

\(M_5\): Amount (mg) of Mexiletine Hydrochloride RS

**Internal standard solution**—A solution of phenetylamine hydrochloride in the mobile phase (3 in 2000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (about 7 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 2.5 g of sodium lauryl sulfate and 3 g of sodium dihydrogenphosphate dihydrate in 600 mL of
water, and add 420 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of mexiletine is about 6 minutes.

Selection of column: Proceed with 20 µL of the standard solution under the above conditions, and calculate the resolution. Use a column giving elution of the internal standard and mexiletine in this order with the resolution between these peaks being not less than 9.

**Containers and storage** Containers—Tight containers.

**Storage**—Light-resistant.

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**Miconazole**

ミコナゾール

![Miconazole structure](image)

C₁₈H₁₄Cl₄N₂O: 416.13
1-[(2RS)-2-(2,4-Dichlorobenzyloxy)-2-(2,4-
dichlorophenyl)ethyl]-1H-imidazole

[Miconazole structure](image)

Miconazole, when dried, contains not less than 98.5% of C₁₈H₁₄Cl₄N₂O.

**Description** Miconazole occurs as a white to pale yellowish white, crystalline powder.

It is freely soluble in methanol, in ethanol (95) and in acetic acid (100), soluble in diethyl ether, and practically insoluble in water.

A solution of Miconazole in methanol (1 in 20) shows no optical rotation.

**Identification (1)** Determine the absorption spectrum of a solution of Miconazole in methanol (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry,<ref>2.25</ref> and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Identification (2)** Determine the infrared absorption spectrum of Miconazole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry,<ref>2.25</ref> and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point**<ref>2.60</ref> 84 – 87°C.

**Purity (1)** Heavy metals <ref>1.07</ref>—Proceed with 1.0 g of Miconazole according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <ref>1.11</ref>—Prepare the test solution with 1.0 g of Miconazole according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Miconazole in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography.<ref>2.05</ref> Spot 50 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, chloroform, methanol and ammonia solution (28:60:30:10:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 20 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying**<ref>2.4d</ref> Not more than 0.5% (1 g, in vacuum, silica gel, 60%, 3 hours).

**Residue on ignition**<ref>2.4d</ref> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Miconazole, previously dried, dissolve in 40 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (indicator: 3 drops of p-naphtholbenzene TS) until the color of the solution changes from light yellow-brown to light yellow-green. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 41.61 mg of C₁₈H₁₄Cl₄N₂O

**Containers and storage** Containers—Tight containers.

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**Miconazole Nitrate**

ミコナゾール硝酸塩

![Miconazole Nitrate structure](image)

C₁₈H₁₄Cl₄N₂O.HNO₃: 479.14
1-[(2RS)-2-(2,4-Dichlorobenzyloxy)-2-(2,4-
dichlorophenyl)ethyl]-1H-imidazole mononitrate

[Miconazole Nitrate structure](image)

Miconazole Nitrate, when dried, contains not less than 98.5% of C₁₈H₁₄Cl₄N₂O.HNO₃.

**Description** Miconazole Nitrate occurs as a white crystalline powder.

It is freely soluble in N,N-dimethylformamide, sparingly soluble in methanol, slightly soluble in ethanol (95), in acetone and in acetic acid (100), and very slightly soluble in water and in diethyl ether.

Melting point: about 180°C (with decomposition).

**Identification (1)** To 2 mL of a solution of Miconazole Nitrate in methanol (1 in 100) add 2 mL of Reinecke salt TS: a light red precipitate is formed.

(2) Determine the absorption spectrum of a solution of Miconazole Nitrate in methanol (1 in 2500) as directed under Thin-layer Chromatography.
Ultraviolet-visible Spectrophotometry <2.4>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Perform the test with a solution of Miconazole Nitrate in methanol (1 in 100) as directed under Flame Coloration Test <1.04> (2); a green color appears.

(4) A solution of Miconazole Nitrate in methanol (1 in 100) responds to the Qualitative Tests <1.09> for nitrate.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Miconazole Nitrate in 100 mL of methanol: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.10 g of Miconazole Nitrate in 6 mL of dilute nitric acid and $N_2N$-dimethylformamide to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and $N_2N$-dimethylformamide to make 50 mL (not more than 0.09%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Miconazole Nitrate according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Miconazole Nitrate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Miconazole Nitrate in 10 mL of methyl alcohol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL, pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 50 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of n-hexane, chloroform, methanol and ammonia solution (28) (60:30:10:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate in iodine vapor for 20 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.4>—Not more than 0.5% (1 g, in vacuum, silica gel, 60°C, 3 hours).

**Residue on ignition** <2.44>—Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.35 g of Miconazole Nitrate, previously dried, dissolve in 30 mL of acetic acid (100) by warming, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 47.91 mg of $C_{18}H_{14}Cl_4N_2O.HNO_3$

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

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**Micronomicin Sulfate**

ミクロノマイシン硫酸塩

$\text{(C}_{20}\text{H}_{41}\text{N}_{5}\text{O}_{7})_2.5\text{H}_{2}\text{SO}_{4}; 1417.53}$

2-Amino-2,3,4,6-tetrahydroxy-6-methylamino-α,D-
erythro-hexopyranosyl-(1→4) [3-deoxy-4-C-methyl-3-
methylamino-β-L-arabinopyranosyl-(1→6)]-2-deoxy-β-
streptamine hemipentasulfate

$[5293-21-7, \text{Micronomicin}]$

Micronomicin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Micronomospora sagamiensis*.

It contains not less than 590 µg (potency) and not more than 660 µg (potency) per mg, calculated on the anhydrous basis. The potency of Micronomicin Sulfate is expressed as mass (potency) of micromonicin ($C_{20}H_{41}N_5O_7$: 463.57).

**Description** Micronomicin Sulfate occurs as a white to light yellowish white powder.

It is very soluble in water, sparingly soluble in ethylene glycol, and practically insoluble in methanol and in ethanol (99.5).

It is hygroscopic.

**Identification** (1) Dissolve 50 mg each of Micronomicin Sulfate and Micronomicin Sulfate RS in 10 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 µL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), 1-butanol and ammonia solution (28) (10:8:7) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in a mixture of acetone and pyridine (25:1) (1 in 500), and heat at 100°C for 10 minutes: the spots obtained from the sample solution and the standard solution are red-purple to red-brown and their $R_f$ values are the same.

(2) To 5 mL of a solution of Micronomicin Sulfate (1 in 100) add 1 mL of barium chloride TS: a white precipitate is formed, and it does not dissolve by addition of dilute nitric acid.

**Optical rotation** <2.4> $[\alpha]_D^20 = +110$ to $+130^\circ$ (0.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).
Midecamycin / Official Monographs

**Midecamycin**

ミデカマイシン

**C₄₁H₆₇NO₁₅**: 813.97

(3R, 9R, 5S, 6R, 8R, 9R, 10E, 12E, 15R)-5, 16-Dideoxy-3-C-methyl-4-O-propanoyl- o-L-ribo-hexopyranosyl(1→4)-3, 6-dideoxy-3-dimethylamino-β-D-glucopyranosylxylo-6-formylmethyl-9-hydroxy-4-methoxy-8-methyl-3-propanoyloxyhexadeca-10, 12-dien-15-olide [35457-80-8]

Midecamycin is a macrolide substance having antibacterial activity produced by the growth of Streptomyces mycarfaciens.

It contains not less than 950 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dried basis. The potency of Midecamycin is expressed as mass (potency) of midecamycin (C₄₁H₆₇NO₁₅).

**Description**

Midecamycin occurs as a white crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (95), and very slightly soluble in water.

**Identification**

(1) Determine the absorption spectrum of a solution of Midecamycin in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Midecamycin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Midecamycin as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Midecamycin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <2.6b> 153 – 158°C.

**Purity**

Heavy metals <1.07>—Proceed with 1.0 g of Midecamycin according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

**Loss on drying** <2.41> Not more than 2.0% (1.0 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.2% (1 g).

**Assay**

Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics.

**Purity (1)**

Clarity and color of solution—Dissolve 1.5 g of Micronomicin Sulfate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Micronomicin Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.40 g of Micronomicin Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.02>. Spot 5 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), 1-butanol and ammonia solution (28) (10:8:7) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in a mixture of acetone and pyridine (25:1) (1 in 500), and heat at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**Water** <2.48> Not more than 10.0% (0.2 g, volumetric titration, back titration). Use a mixture of methanol for water determination and ethylene glycol for water determination (1:1) instead of methanol for water determination.

**Assay**

Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—Bacillus subtilis ATCC 6633

(ii) Culture medium—Use the medium in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Micronomicin Sulfate RS, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 10°C, and use within 30 days. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 2 μg (potency) and 0.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Micronomicin Sulfate, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 20 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 2 μg (potency) and 0.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

**Containers and storage**

Containers—Tight containers.
Midecamycin Acetate

ミデカマイシン酢酸エステル

\[
\mathrm{C_{45}H_{71}NO_{17}}: 898.04 \\
(3R,4S,5S,6R,8R,9R,10E,12E,15R)-9-Acetoxy-5-[3-O-acetyl-2,6-dideoxy-3-C-methyl-4-O-propanoyl-\alpha-L-ribo-hexopyranosyl-(1\rightarrow4)]-3,6-dideoxy-3-dimethylamino-\beta-D-glucopyranosyloxy]-6-formylmethyl-4-methoxy-8-methyl-3-propioloyxyhexadeca-10,12-dien-15-olide \\
[53881-07-7]
\]

Midecamycin Acetate is a derivative of midecamycin.

It contains not less than 950 \(\mu g\) (potency) and not more than 1010 \(\mu g\) (potency) per mg, calculated on the dried basis. The potency of Midecamycin Acetate is expressed as mass of midecamycin acetate \((\mathrm{C_{45}H_{71}NO_{17}})\).

Description Midecamycin Acetate occurs as white, crystals or crystalline powder.

It is sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Midecamycin Acetate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\rangle\), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Midecamycin Acetate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Midecamycin Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\rangle\), and compare the spectrum with the Reference Spectrum or spectrum of dried Midecamycin Acetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity Heavy metals \(<\ 0.07\rangle\)—Proceed with 1.0 g of Midecamycin Acetate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying \(<2.41\rangle\) Not more than 2.0% (1 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition \(<2.44\rangle\) Not more than 0.2% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics \(<4.02\rangle\) according to the following conditions.

(i) Test organism—\textit{Micrococcus luteus} ATCC 9341

(ii) Culture medium—Use the medium in 3) Medium for other organisms under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Midecamycin Acetate RS, previously dried, equivalent to about 25 mg (potency), and dissolve in methanol to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 – 15°C and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 \(\mu g\) (potency) and 5 \(\mu g\) (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Midecamycin, previously dried, equivalent to about 20 mg (potency), dissolve in 10 mL of methanol, add water to make exactly 50 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 \(\mu g\) (potency) and 5 \(\mu g\) (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.
Migrenin

ミグレニン

Migrenin is composed of 90 parts of antipyrine, 9 parts of caffeine, and 1 part of citric acid in mass.

Migrenin, when dried, contains not less than 87.0% and not more than 93.0% of antipyrine (C₁₁H₁₂N₂O: 188.23) and not less than 8.6% and not more than 9.5% of caffeine (C₈H₁₀N₄O₂: 194.19).

**Description** Migrenin occurs as a white powder or crystaline powder. It is odorless and has a bitter taste.

It is very soluble in water, freely soluble in ethanol (95) and in chloroform, and slightly soluble in diethyl ether.

The pH of a solution of Migrenin (1 in 10) is between 3.0 and 4.0.

It is affected by moisture and light.

**Identification**

1. To 5 mL of a solution of Migrenin (1 in 100) add 2 drops of sodium nitrite TS and 1 mL of dilute sulfuric acid: a deep green color develops.

2. To 5 mL of a solution of Migrenin (1 in 50) add 1 drop of hydrochloric acid and 0.2 mL of formaldehyde solution, heat in a water bath for 30 minutes, add an excess of ammonia TS, and filter. Acidify the filtrate with hydrochloric acid, shake with 3 mL of chloroform, and separate the chloroform layer. Evaporate the chloroform solution on a water bath, add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid to the residue, and evaporate on a water bath to dryness: the residue shows a yellow-red color.

3. A solution of Migrenin (1 in 10) responds to the Qualitative Tests for citrate.

**Melting point** 104 – 110°C.

**Purity**

1. Clarity and color of solution—Dissolve 1.0 g of Migrenin in 40 mL of water: the solution is clear and colorless to pale yellow.

2. Heavy metals—Proceed with 1.0 g of Migrenin according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

**Residue on ignition** Not more than 0.1% (1 g).

**Assay**

1. Antipyrine—Weigh accurately about 0.25 g of Migrenin, previously dried in an iodine flask, dissolve in 25 mL of sodium acetate TS, add exactly 30 mL of 0.05 mol/L iodine VS, and allow to stand for 20 minutes with occasional shaking. Add 15 mL of chloroform to dissolve the precipitate so obtained, and titrate the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

   Each mL of 0.05 mol/L iodine VS = 9.411 mg of C₁₁H₁₂N₂O

2. Caffeine—To about 1 g of Migrenin, previously dried and accurately weighed, add exactly 5 mL of the internal standard solution, dissolve in chloroform to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 90 mg of Caffeine RS, previously dried at 80°C for 4 hours, add exactly 5 mL of the internal standard solution, dissolve in chloroform to make 10 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, $Q_1$ and $Q_9$, of the peak area of caffeine to that of the internal standard.

   Amount (mg) of caffeine (C₈H₁₀N₄O₂) = $M_8 \times Q_1/Q_9$

   $M_8$: Amount (mg) of Caffeine RS

**Internal standard solution**—A solution of ethenzamide in chloroform (1 in 50).

**Operating conditions**

Detector: A hydrogen flame-ionization detector.

Column: A glass column 2.6 mm in inside diameter and 210 cm in length, packed with silicone earth for gas chromatography (180 to 250 μm in particle diameter) coated with 50% phenyl-methyl silicon polymer for gas chromatography at the ratio of 15%.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of ethenzamide is about 4 minutes.

**System suitability**

System performance: Dissolve 0.9 g of antipyrine and 0.09 g of caffeine in 10 mL of chloroform. When the procedure is run with 1 μL of this solution under the above operating conditions, caffeine and antipyrine are eluted in this order with the relative standard deviation of the ratios of the peak area of caffeine to that of the internal standard not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.
Minocycline Hydrochloride

ミノサイクリン塩酸塩

C₂₃H₂₇N₃O₇·HCl: 493.94

(4S,4aS,5aR,12aS)-4,7-Bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide monohydrochloride [13614-98-7]

Minocycline Hydrochloride is the hydrochloride of a derivative of tetracycline. It contains not less than 890 μg (potency) and not more than 950 μg (potency) per mg, calculated on the anhydrous basis. The potency of Minocycline Hydrochloride is expressed as mass (potency) of minocycline (C₂₃H₂₇N₃O₇): 457.48.

Description Minocycline Hydrochloride occurs as a yellow crystalline powder. It is freely soluble in N,N-dimethylformamide, soluble in methanol, sparingly soluble in water, and slightly soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Minocycline Hydrochloride in a solution of hydrochloric acid in methanol (19 in 20,000) (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Minocycline Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Minocycline Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.23>, and compare the spectrum with the Reference Spectrum or the spectrum of Minocycline Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Minocycline Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> (2) for chloride.

pH <2.5>< (3) Related substances—Dissolve 50 mg of Minocycline Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Perform the test immediately after the preparation of the sample solution with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of each peak area by the area percentage method: the amount of epiminocycline is not more than 1.2%, the amount of each peak other than minocycline and epiminocycline is not more than 1.0%, and the total area of the peaks other than minocycline and epiminocycline is not more than 2.0%.

Operating conditions—

Detector, column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay. Flow rate: Adjust the flow rate so that the retention time of minocycline is about 12 minutes. The retention time of epiminocycline is about 10 minutes under this condition. Time span of measurement: About 2.5 times as long as the retention time of minocycline beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the sample solution add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of minocycline obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that from 20 μL of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of minocycline is not more than 2.0%.

Water <2.48>< Not less than 4.3% and not more than 8.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.5% (1 g).

Assay Weigh accurately an amount of Minocycline Hydrochloride and Minocycline Hydrochloride RS, equivalent to about 50 mg (potency), dissolve each in the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A₁ and A₃, of minocycline of these solutions.

Amount [μg (potency)] of minocycline (C₂₃H₂₇N₃O₇) = Mₛ × A₁/A₃ × 1000

Mₛ: Amount [mg (potency)] of Minocycline Hydrochloride RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm). Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter). Column temperature: A constant temperature of about
Minocycline Hydrochloride for Injection

Minocycline Hydrochloride for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of minocycline (C23H27N3O7: 457.48).

Method of preparation

Prepare as directed under Injections, with Minocycline Hydrochloride.

Description

Minocycline Hydrochloride for Injection occurs as a yellow to yellow-brown powder or flakes.

Identification

Dissolve 4 mg of Minocycline Hydrochloride for Injection in 250 mL of a solution of hydrochloric acid in methanol (19 in 20,000). The absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24> exhibits maxima between 221 nm and 225 nm, between 261 nm and 265 nm, and between 354 nm and 358 nm.

pH <2.54> The pH of a solution, prepared by dissolving an amount of Minocycline Hydrochloride for Injection, equivalent to 0.1 g (potency) of Minocycline Hydrochloride according to the labeled amount, in 10 mL of water is 2.0 to 3.5.

Purity

Related substances—Conduct this procedure rapidly after the preparation of the sample solution. Take an amount of Minocycline Hydrochloride for Injection, equivalent to 0.1 g (potency) of Minocycline Hydrochloride according to the labeled amount, dissolve in the mobile phase to make 100 mL. To 25 mL of this solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amounts of each peak by the area percentage method: the amount of epi-minocycline, having the relative retention time of about 0.83 with respect to minocycline, is not more than 6.0%.

Operating conditions—

Detector, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of minocycline, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution obtained in the Assay, add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add the mobile phase to make exactly 100 mL. Confirm that the peak area of minocycline obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that from 20 μL of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of peak areas of minocycline is not more than 1.0%.

Containers and storage

Containers—Tight containers.

Storage—Light-resistant.

Minocycline Hydrochloride for Injection

注射用ミノサイクリン塩酸塩

Minocycline Hydrochloride for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of minocycline (C23H27N3O7: 457.48).

Method of preparation

Prepare as directed under Injections, with Minocycline Hydrochloride.

Description

Minocycline Hydrochloride for Injection occurs as a yellow to yellow-brown powder or flakes.

Identification

Dissolve 4 mg of Minocycline Hydrochloride for Injection in 250 mL of a solution of hydrochloric acid in methanol (19 in 20,000). The absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24> exhibits maxima between 221 nm and 225 nm, between 261 nm and 265 nm, and between 354 nm and 358 nm.

pH <2.54> The pH of a solution, prepared by dissolving an amount of Minocycline Hydrochloride for Injection, equivalent to 0.1 g (potency) of Minocycline Hydrochloride according to the labeled amount, in 10 mL of water is 2.0 to 3.5.

Purity

Related substances—Conduct this procedure rapidly after the preparation of the sample solution. Take an amount of Minocycline Hydrochloride for Injection, equivalent to 0.1 g (potency) of Minocycline Hydrochloride according to the labeled amount, dissolve in the mobile phase to make 100 mL. To 25 mL of this solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amounts of each peak by the area percentage method: the amount of epi-minocycline, having the relative retention time of about 0.83 with respect to minocycline, is not more than 6.0%.

Operating conditions—

Detector, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of minocycline, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution obtained in the Assay, add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add the mobile phase to make exactly 100 mL. Confirm that the peak area of minocycline obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that from 20 μL of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of minocycline is not more than 2.0%.

Water <2.48> Weigh accurately the mass of the content of one container of Minocycline Hydrochloride for Injection, dissolve in exactly 2 mL of methanol for water determination, and perform the test with exactly 1 mL of this solution as directed in the Volumetric titration (back titration): not more than 3.0%.

Bacterial endotoxins <4.01> Less than 1.25 EU/mg (potency).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay

Weigh accurately the mass of the contents of not less than 10 containers of Minocycline Hydrochloride for Injection. Weigh accurately an amount of the contents, equivalent to about 0.1 g (potency) of Minocycline Hydrochloride, dissolve in the mobile phase to make exactly 100 mL. Pipet 25 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Minocycline Hydrochloride RS, equivalent to about 25 mg (potency), dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Minocycline Hydrochloride.

Amount [mg (potency)] of minocycline (C23H27N3O7) = M5 × A1/A5 × 4

M5: Amount [mg (potency)] of Minocycline Hydrochlo-
Containers and storage  Containers—Hermetic containers.

Minocycline Hydrochloride Tablets

ミノサイクリン塩酸塩錠

Minocycline Hydrochloride Tablets contain not less than 90.0% and not more than 110.0% of the labeled potency of Minocycline (C_{23}H_{27}N_{3}O_{7}: 457.48).

Method of preparation  Prepare as directed under Tablets, with Minocycline Hydrochloride.

Identification  To a quantity of powdered Minocycline Hydrochloride Tablets, equivalent to 10 mg (potency) of Minocycline Hydrochloride according to the labeled amount, add 625 mL of a solution of hydrochloric acid in methanol (19 in 20,000), shake well, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24> it exhibits maxima between 221 nm and 225 nm, between 261 nm and 265 nm, and between 354 nm and 358 nm.

Purity  Related substances—Conduct this procedure rapidly after preparation of the sample solution. Powder not less than 5 Minocycline Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to 50 mg (potency) of Minocycline Hydrochloride according to the labeled amount, add 60 mL of the mobile phase, shake vigorously, and add the mobile phase to make 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.0D> according to the following conditions. Determine each peak area by the automatic integration method. Calculate the amounts of these peaks by the area percentage method: the amount of the peak of epiminocycline, having the relative retention time of about 0.83 with respect to minocycline, is not more than 2.0%.

Operating conditions—
Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.
Time span of measurement: About 2.5 times as long as the retention time of minocycline, beginning after the solvent peak.
System suitability—
Test for required detectability: To 2 mL of the sample solution add the mobile phase to make 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of minocycline obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that of minocycline from 20 μL of the solution for system suitability test.
System performance: Proceed as directed in the system suitability in the Assay.
System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of minocycline is not more than 2.0%.

Water <2.48> Not more than 12.0% (0.5 g of powdered Minocycline Hydrochloride Tablets, volumetric titration, back titration).

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.
To 1 tablet of Minocycline Hydrochloride Tablets add 60 mL of the mobile phase, treat with ultrasonic waves for 15 minutes, and add the mobile phase to make exactly V mL so that each mL contains about 0.5 mg (potency) of Minocycline Hydrochloride. Centrifuge this solution, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.
Amount [mg (potency)] of minocycline (C_{23}H_{27}N_{3}O_{7})
= M_5 × A_5/T × V/50

M_5: Amount [mg (potency)] of Minocycline Hydrochloride RS
Dissolution <6.10D> When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Minocycline Hydrochloride Tablets is not less than 85%.
Start the test with 1 tablet of Minocycline Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 9 μg (potency) of Minocycline Hydrochloride according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately an amount of Minocycline Hydrochloride RS, equivalent to about 30 mg (potency), and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S, at 348 nm.

Dissolution rate (%) with respect to the labeled amount of minocycline (C_{23}H_{27}N_{3}O_{7})
= M_5 × A_T/A_S × V/V' × 1/C × 36

M_5: Amount [mg (potency)] of Minocycline Hydrochloride RS
C: Labeled amount [mg (potency)] of minocycline (C_{23}H_{27}N_{3}O_{7}) in 1 tablet

Assay  To a number of Minocycline Hydrochloride Tablets, equivalent to about 1 g (potency) of Minocycline Hydrochloride, add 120 mL of the mobile phase, treat with ultrasonic waves for 15 minutes, and add the mobile phase to make exactly 200 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Minocycline Hydrochloride RS, equivalent to about 25 mg (potency), dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Minocycline Hydrochloride.
Mitomycin C

Mitomycin C is a substance having antitumor activity produced by the growth of Streptomyces caesipitosus.

It contains not less than 970 μg (potency) and not more than 1030 μg (potency) per mg, calculated on the dried basis. The potency of Mitomycin C is expressed as mass (potency) of mitomycin C (C₁₅H₁₈N₄O₅).

**Description** Mitomycin C occurs as blue-purple, crystals or crystalline powder.

It is freely soluble in N,N-dimethylacetamide, slightly soluble in water and in methanol, and very slightly soluble in ethanol (99.5).

**Identification (1)** Determine the absorption spectrum of a solution of Mitomycin C (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 2.2.24, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mitomycin C RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mitomycin C as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.2.25, and compare the spectrum with the Reference Spectrum or the spectrum of Mitomycin C RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** Related substances—Conduct this procedure rapidly after the sample and the standard solutions are prepared. Dissolve 50 mg of Mitomycin C in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.2.01, according to the following conditions, and determine each peak area by the automatic integration method: each area of the peak other than mitomycin C obtained from the sample solution is not larger than the peak area of mitomycin C from the standard solution, and the total area of the peaks other than mitomycin C from the sample solution is not larger than 3 times the peak area of mitomycin C from the standard solution.

**Operating conditions—**

- **Detector:** An ultraviolet absorption photometer (wavelength: 254 nm).
- **Column:** A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- **Column temperature:** A constant temperature of about 30°C.
- **Mobile phase A:** To 20 mL of 0.5 mol/L ammonium acetate TS add water to make 1000 mL. To 800 mL of this solution add 200 mL of methanol.
- **Mobile phase B:** To 20 mL of 0.5 mol/L ammonium acetate TS add water to make 1000 mL. To this solution add 1000 mL of methanol.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>10 – 30</td>
<td>100 → 0</td>
<td>0 → 100</td>
</tr>
<tr>
<td>30 – 45</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Flow rate: About 1.0 mL per minute.

Time span of measurement: About 2 times as long as the retention time of mitomycin C beginning after the solvent peak.

**System suitability—**

Test for required detection: Pipet 10 mL of the standard solution, and add methanol to make exactly 100 mL. Confirm that the peak area of mitomycin C obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: Dissolve 25 mg of Mitomycin C and 40 mg of 3-ethoxy-4-hydroxybenzaldehyde in 50 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions, mitomycin C and 3-ethoxy-4-hydroxybenzaldehyde are eluted in this order with the resolution between these peaks being not less than 15.

System repeatability: When the test is repeated 3 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mitomycin C is not more than 3.0%.

**Loss on drying** Not more than 1.0% (0.1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

**Assay** Weigh accurately an amount of Mitomycin C and Mitomycin C RS, equivalent to about 25 mg (potency), dissolve each in N,N-dimethylacetamide to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.2.01, according to the fol-
Mitomycin C for Injection

Mitomycin C for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of mitomycin C (C\textsubscript{15}H\textsubscript{18}N\textsubscript{4}O\textsubscript{5} \text{H\textsubscript{2}O}\times 0.5 \text{g}, in vacuo) and not exceeding 0.67 kPa, phosphorus (V) oxide, 60°C, 3 hours).

Bacterial endotoxins Less than 10 EU/mg (potency).

Uniformity of dosage units Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 container of Mitomycin C for Injection add exactly V mL of N,N-dimethylacetamide so that each mL contains about 0.5 mg (potency) of Mitomycin C, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg (potency) of Mitomycin C RS, add N,N-dimethylacetamide to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Mitomycin C.

Assay Weigh accurately the mass of the contents of not less than 10 containers of Mitomycin C for Injection. Weigh accurately an amount of the contents, equivalent to about 10 mg (potency) of Mitomycin C, add exactly 20 mL of N,N-dimethylacetamide, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately an amount of Mitomycin C RS, equivalent to about 25 mg (potency), dissolve in N,N-dimethylacetamide to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Mitomycin C.

Containers and storage Containers—Hermetic containers.

Mizoribine

\[
\text{C}_9\text{H}_{13}\text{N}_3\text{O}_6: 259.22
\]

5-Hydroxy-1-β-D-ribofuranosyl-1H-imidazole-4-carboxamide [50024-49-7]

Mizoribine contains not less than 98.0% and not more than 102.0% of C\textsubscript{9}H\textsubscript{13}N\textsubscript{3}O\textsubscript{6}, calculated on the anhydrous basis.
Description Mizoribine occurs as a white to yellowish white crystalline powder.

It is freely soluble in water, and practically insoluble in methanol and in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Mizoribine (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mizoribine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mizoribine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Mizoribine RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20} - 25 - -27^\circ$ (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Mizoribine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Mizoribine in the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 5 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of mizoribine, $A_1$ and $A_2$, of both solutions.

Amount (mg) of $\text{C}_9\text{H}_{13}\text{N}_3\text{O}_6 = M_S \times A_1/A_2 \times 10$

$M_S$: Amount (mg) of Mizoribine RS, calculated on the anhydrous basis.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 279 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadeclsilanized silica gel for liquid chromatography (5 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Diluted phosphoric acid (1 in 1500).

Flow rate: Adjust the flow rate so that the retention time of mizoribine is about 9 minutes.

System suitability—

System performance: When the procedure is run with 5 $\mu$L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mizoribine are not less than 10,000 and not more than 1.4, respectively.

System repeatability: When the test is repeated 6 times with 5 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mizoribine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Mizoribine Tablets

ミゾリビン錠

Mizoribine Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of mizoribine ($\text{C}_9\text{H}_{13}\text{N}_3\text{O}_6$: 259.22).

Method of preparation Prepare as directed under Tablets, with Mizoribine.

Identification To a quantity of powdered Mizoribine Tablets, equivalent to 0.1 g of Mizoribine according to the labeled amount, add 5 mL of water, shake, filter, and use the filtrate as the sample solution. Separately, dissolve 20 mg of Mizoribine RS in 1 mL of water, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Thin-Layer Chromatography <2.03>. Spot 1 $\mu$L each of the sample solu-
tion and standard solution on a plate of silica gel for thin-layer chromatography. Then develop the plate with a mixture of methanol, ammonia solution (28) and 1-propanol (2:1:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the principal spot from the sample solution and the spot from the standard solution show a red-brown color and the same Rf value.

**Purity** Related substances—To a quantity of powdered Mizoribine Tablets, equivalent to 0.10 g of Mizoribine according to the labeled amount, add 30 mL of the mobile phase, shake, then add the mobile phase to make 50 mL. Filter the solution through a membrane filter with a pore size not exceeding 0.5 μm and use the filtrate as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 20 mL. Filter the solution through a membrane filter with a pore size not exceeding 0.5 μm and use the filtrate as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of each solution by the automatic integration method: the area of the peak, having the relative retention time of about 0.3 with respect to mizoribine, obtained from the sample solution is not larger than the peak area of mizoribine from the standard solution, and the area of the peak other than mizoribine and other than the peak mentioned above is not larger than 2/5 times the peak area of mizoribine from the standard solution.

**Operating conditions—**

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Mizoribine.

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Time span of measurement: About 3 times as long as the retention time of mizoribine, beginning after the solvent peak.

**System suitability—**

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 5 mL. Confirm that the peak area of mizoribine obtained from 5 μL of this solution is equivalent to 14 to 26% of that from 5 μL of the standard solution.

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mizoribine are not less than 10,000 and not more than 1.4, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mizoribine is not more than 2.0%.

**Uniformity of dosage units <6.02>** Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Mizoribine Tablets add 50 mL of water, shake until the tablet is disintegrated, and add water to make exactly 100 mL. Filter the solution, discard not less than 10 mL of the first filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 5 μg of mizoribine (C9H13N3O6), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Mizoribine Tablets (separately determine the water <2.48> in the same manner as Mizoribine), and dissolve in water to make exactly 100 mL. Pipet 2 mL of the solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A1 and A5, at 279 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

**Amount of mizoribine (C9H13N3O6) = M5 × A1/A5 × V/V × 1/50**

**Dissolution <6.10>** When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Mizoribine Tablets is not less than 80%.

Start the test with 1 tablet of Mizoribine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard not less than 10 mL of the first filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V mL so that each mL contains about 14 μg of mizoribine (C9H13N3O6) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Mizoribine RS (separately determine the water <2.48> in the same manner as Mizoribine), and dissolve in water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A1 and A5, at 279 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

**Dissolution rate (%) with respect to the labeled amount of mizoribine (C9H13N3O6) = M5 × A1/A5 × V/V × 1/C × 45**

**Containers and storage** Containers—Tight containers.

**Official Monographs / Mizoribine Tablets 1129**
Morphine and Atropine Injection

モルヒネ・アトロピン注射液

Morphine and Atropine Injection is an aqueous solution for injection.

It contains not less than 0.91 w/v% and not more than 1.09 w/v% of morphine hydrochloride hydrate (C_{17}H_{23}NO_{3}.HCl.3H_{2}O: 375.84), and not less than 0.027 w/v% and not more than 0.033 w/v% of atropine sulfate hydrate [(C_{17}H_{23}NO_{3})_2.H_2SO_{4}.H_2O: 694.83].

Method of preparation

<table>
<thead>
<tr>
<th>Morphin Hydrochloride Hydrate</th>
<th>10 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine Sulfate Hydrate</td>
<td>0.3 g</td>
</tr>
<tr>
<td>Water for Injection or Sterile Water</td>
<td>a significant quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

Description

Morphine and Atropine Injection is a clear, colorless liquid.

pH: 2.5 ~ 5.0

Identification

To 2 mL of Morphine and Atropine Injection add 2 mL of ammonia TS, and extract with 10 mL of diethyl ether. Filter the extract with a filter paper, evaporate the filtrate on a water bath to dryness, dissolve the residue in 1 mL of ethanol (99.5), and use this solution as the sample solution. Separately, dissolve 0.1 g of morphine hydrochloride hydrate in 10 mL of water, perform with 2 mL of this solution the same procedure as used for preparation of the sample solution, and use the solution so obtained as the standard solution (1). Separately, dissolve 3 mg of atropine sulfate in 10 mL of water, perform with 2 mL of this solution the same procedure as used for preparation of the sample solution, and use the solution so obtained as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography. Develop the plate with a mixture of methyl and ammonia solution (28:200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff’s TS on the plate: the two spots obtained from the sample solution show the same color tone and the same Rf value with either spot of orange color obtained from the standard solution (1) or the standard solution (2) (morphine and atropine).

Extractable volume <6.0> It meets the requirement.

Assay

(1) Morphine hydrochloride hydrate—Pipet 2 mL of Morphine and Atropine Injection, add exactly 10 mL of the internal standard solution, then add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, add exactly 10 mL of the internal standard solution to dissolve, then add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of morphine to that of the internal standard.

\[
Q_T = \frac{M_S}{M} 
\]

Amount (mg) of morphine hydrochloride hydrate

\[
(C_{17}H_{23}NO_{3}.HCl.3H_2O) = M_S \times Q_T/Q_S \times 1.168
\]

M_S: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH with sodium hydroxide TS to 3.0. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

(2) Atropine sulfate hydrate—Pipet 2 mL of Morphine and Atropine Injection, add exactly 2 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of Atropine Sulfate RS (separately determine the loss on drying <2.4>) under the same conditions as Atropine Sulfate Hydrate, and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak areas of atropine to that of the internal standard.

\[
Q_S = \frac{M_S}{M} 
\]

Amount (mg) of atropine sulfate hydrate

\[
[(C_{17}H_{23}NO_{3})_2.H_2SO_{4}.H_2O] = M_S \times Q_T/Q_S \times 1/25 \times 1.027
\]

M_S: Amount (mg) of Atropine Sulfate RS, calculated on the dried basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 12,500).

Operating conditions—

Column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay (1).
Detector: An ultraviolet absorption photometer (wavelength: 225 nm).
Flow rate: Adjust the flow rate so that the retention time of morphine is about 7 minutes.

System suitability—
System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, morphine, the internal standard and atropine are eluted in this order, and the resolution between morphine and the internal standard is not less than 3.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of atropine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

Morphine Hydrochloride Hydrate

Morphine Hydrochloride Hydrate contains not less than 98.0% and not more than 102.0% of morphine hydrochloride (C₁₇H₁₉NO₃.HCl: 321.80), calculated on the anhydrous basis.

Description Morphine Hydrochloride Hydrate occurs as white, crystals or crystalline powder.
It is freely soluble in formic acid, soluble in water, sparingly soluble in methanol, and slightly soluble in ethanol (95).
It gradually becomes yellow-brown by light.

Identification (1) Determine the absorption spectrum of a solution of Morphine Hydrochloride Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Morphine Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum.

(3) A solution of Morphine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests 1.09 for chloride.

Optical rotation $\leq 2.4^\circ$: $[\alpha]_{D}^{25} = -111 - -116^\circ$ (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH $\leq 2.5^\circ$ The pH of a solution obtained by dissolving 0.10 g of Morphine Hydrochloride Hydrate in 10 mL of water is between 4.0 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 0.40 g of Morphine Hydrochloride Hydrate in 10 mL of water; the solution is clear. When perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry 2.24, the absorbance at 420 nm is not more than 0.12.

(2) Sulfate $\leq 2.4^\circ$—Dissolve 0.20 g of Morphine Hydrochloride Hydrate in 5 mL of water, and add 2 to 3 drops of barium chloride TS: no turbidity is produced.

(3) Meconic acid—Dissolve 0.20 g of Morphine Hydrochloride Hydrate in 5 mL of water, and add 5 mL of dilute hydrochloric acid and 2 drops of iron (III) chloride TS: no red color develops.

(4) Related substances—Dissolve 0.1 g of Morphine Hydrochloride Hydrate in 10 mL of diluted ethanol (95) (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ethanol (95) (1 in 2) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.03. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), toluene, acetone and ammonia solution (28) (14:14:7:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water $\leq 2.4^\circ$ 13 – 15% (0.1 g, direct titration).

Residue on ignition $\leq 2.4^\circ$ Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.5 g of Morphine Hydrochloride Hydrate, dissolve in 3.0 mL of formic acid, add 100 mL of a mixture of acetic anhydride and acetic acid (100); mix, and titrate $\leq 2.5^\circ$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS $= 32.18$ mg of C₁₇H₁₉NO₃.HCl

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Morphine Hydrochloride Injection

Morphine Hydrochloride Injection is an aqueous solution for injection.
It contains not less than 93.0% and not more than 107.0% of the labeled amount of morphine hydrochlo-
Morphine Hydrochloride Tablets

Method of preparation
Prepare as directed under Tablets, with Morphine Hydrochloride Hydrate.

Identification
Weigh a quantity of powdered Morphine Hydrochloride Tablets equivalent to 0.01 g of Morphine Hydrochloride Hydrate, add 100 mL of water, shake for 10 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24> it exhibits a maximum between 283 nm and 287 nm. And weigh a quantity of powdered Morphine Hydrochloride Tablets equivalent to 0.01 g of Morphine Hydrochloride Hydrate, add 100 mL of dilute sodium hydroxide TS, shake for 10 minutes, and filter. Determine the absorption spectrum of the filtrate: it exhibits a maximum between 296 nm and 300 nm.

System suitability—
System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

Containers and storage
Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

Morphine Hydrochloride Tablets

モルヒネ塩酸塎

Morphine Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of morphine hydrochloride hydrate (C_{17}H_{19}NO_{3}.HCl.3H_{2}O: 375.84).

Method of preparation
Prepare as directed under Tablets, with Morphine Hydrochloride Hydrate.

Identification
Weigh a quantity of powdered Morphine Hydrochloride Tablets equivalent to 0.01 g of Morphine Hydrochloride Hydrate, add 100 mL of water, shake for 10 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24> it exhibits a maximum between 283 nm and 287 nm. And weigh a quantity of powdered Morphine Hydrochloride Tablets equivalent to 0.01 g of Morphine Hydrochloride Hydrate, add 100 mL of dilute sodium hydroxide TS, shake for 10 minutes, and filter. Determine the absorption spectrum of the filtrate: it exhibits a maximum between 296 nm and 300 nm.

Uniformity of dosage units
Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Morphine Hydrochloride Tablets add exactly 1 mL of the internal standard solution per 2 mg of morphine hydrochloride hydrate (C_{17}H_{19}NO_{3}.HCl.3H_{2}O: 375.84). Filter the solution, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of morphine hydrochloride hydrate

\[
M_{S} = M_{S} \times \frac{Q_{T}}{Q_{S}} \times 4 \times 1.168
\]

\(M_{S}\): Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis.
internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Dissolution 6.10 When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Morphine Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Morphine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately a quantity of the powder, equivalent to about 20 mg of morphine hydrochloride for assay (separately, determine the water content as directed in the assay for Morphine Hydrochloride Tablets). Weigh accurately about 28 mg of morphine hydrochloride for assay (separately, determine the water content), and dissolve in water to make exactly 50 mL, and use this solution as the standard solution. Per- form the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01, according to the following conditions, and determine the peak areas, $A_T$ and $A_S$, of morphine in each solution.

Dissolution rate (%) with respect to the labeled amount of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$) is not more than 3.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of morphine are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

Containers and storage—Tight containers.

Storage—Light-resistant.

Mosapride Citrate Hydrate

モサプリドクエン酸塩水和物

\[
\begin{align*}
C_{21}H_{25}ClFN_3O_3.C_6H_8O_7.2H_2O: & \quad 650.05 \\
4-Amino-5-chloro-2-ethoxy-N\text{-}\left[\text{(2RS)}\right]4-(4-fluorobenzyl)morpholin-2-yl\text{-}N\text{-}dimethylformamide monocitrate dihydrate \quad [63658-02-2] \\
C_13H_{25}ClF_3N_3O_3.C_6H_8O_7.2H_2O: & \quad 650.05 \\
4-Amino-5-chloro-2-ethoxy-N\text{-}4\text{-}(4-fluorobenzyl)morpholin-2-yl\text{-}N\text{-}dimethylformamide monocitrate dihydrate \\
& \quad [63658-02-2] \\
\end{align*}
\]

Mosapride Citrate Hydrate contains not less than 98.5% and not more than 101.0% of mosapride citrate ($C_{21}H_{25}ClF_3N_3O_3.C_6H_8O_7.2H_2O$). Mosapride Citrate Hydrate occurs as a white to yellowish white crystalline powder.

It is freely soluble in $N,N$-dimethylformamide and in acetone (100), sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

Assay—Take not less than 20 Morphine Hydrochloride Tablets, weigh accurately, and powder. Weigh accurately a quantity of the powder, equivalent to about 20 mg of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$), add exactly 10 mL of the internal standard solution, extract the mixture with ultrasonic waves for 10 minutes, and add water to make 50 mL. Filter this solution, and use the filtrate as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01, according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of morphine to that of the internal standard.

\[
M_S = \frac{S}{A_T/A_S} \times \frac{1}{C} \times 36 \times 1.168
\]

\[
M_S: \text{Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis}
\]

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).
A solution of Mosapride Citrate Hydrate in N,N-dimethylformamide (1 in 20) shows no optical rotation.

**Identification (1)** Determine the absorption spectrum of a solution of Mosapride Citrate Hydrate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mosapride Citrate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Mosapride Citrate Hydrate in N,N-dimethylformamide (1 in 10) responds to the Qualitative Tests <1.09> (1) for citrate.

**Purity (1)** Heavy metals <1.07>—Proceed with 1.0 g of Mosapride Citrate Hydrate in a platinum crucible according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Mosapride Citrate Hydrate in 50 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 50 mL. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 5 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak having the relative retention time of about 0.47 with respect to mosapride from the sample solution is not larger than 3 times the peak area of mosapride from the standard solution, and the area of each peak other than the peak of mosapride and other than the peak mentioned above from the sample solution is not larger than the peak area of mosapride from the standard solution. Furthermore, the total area of the peaks other than the peak of mosapride from the sample solution is not larger than 5 times the peak area of mosapride from the standard solution.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wave-length: 274 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeckylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: Dissolve 8.82 g of trisodium citrate dihydrate in 800 mL of water, adjust the pH to 4.0 with dilute hydrochloric acid, and add water to make 1000 mL.

Mobile phase B: Acetonitrile.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 35</td>
<td>80 → 45</td>
<td>20 → 55</td>
</tr>
</tbody>
</table>

Flow rate: 1.0 mL per minute.

Time span of measurement: Beginning after the solvent peak to 35 minutes after injection.

**System suitability**—

Test for required detectability: Pipet 4 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of mosapride obtained from 5 µL of this solution is equivalent to 15 to 25% of that of mosapride from 5 µL of the standard solution.

System performance: When the procedure is run with 5 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mosapride are not less than 40,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mosapride is not more than 5.0%.

(3) Residual solvent—Being specified separately.

**Water <2.48>** 5.0 – 6.5% (0.5 g, volumetric titration, back titration).

**Residue on ignition <2.44>** Not more than 0.1% (1 g, platinum crucible).

**Assay** Weigh accurately 0.5 g of Mosapride Citrate Hydrate, dissolve in 70 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 61.40 mg of C$_{21}$H$_{25}$ClFN$_3$O$_3$.C$_6$H$_8$O$_7$.

**Containers and storage** Containers—Well-closed containers.

**Mosapride Citrate Powder**

モサプリドクエン酸塩散

Mosapride Citrate Powder contains not less than 93.0% and not more than 107.0% of the labeled amount of mosapride citrate (C$_{21}$H$_{25}$ClFN$_3$O$_3$.C$_6$H$_8$O$_7$: 614.02).

**Method of preparation** Prepare as directed under Granules or Powders, with Mosapride Citrate Hydrate.

**Identification (1)** Powder Mosapride Citrate Powder. To a portion of the powder, equivalent to 10 mg of mosapride citrate (C$_{21}$H$_{25}$ClFN$_3$O$_3$.C$_6$H$_8$O$_7$) according to the labeled amount, add 10 mL of dilute acetic acid, shake for 10 minutes, and filter. To 5 mL of the filtrate add 0.3 mL of Dragendorff’s TS: an orange precipitate is formed.

(2) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima be-
between 271 nm and 275 nm and between 306 nm and 310 nm.

**Purity** Related substances—Powder Mosapride Citrate Powder. To a portion of the powder, equivalent to 10 mg of mosapride citrate (C₂₁H₂₅ClFN₃O₇.C₆H₈O₇) according to the labeled amount, moisten with 1 mL of water, then add 9 mL of methanol, shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 2 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the two peaks, having the relative retention time of about 0.60 and about 0.85 with respect to mosapride obtained from the sample solution, is not larger than the peak area of mosapride from the standard solution, the area of other than the peak of mosapride and the peaks mentioned above is not larger than 2/5 times the peak area of mosapride from the standard solution, and the total area of the peak other than mosapride is not larger than 2 times the peak area of mosapride from the standard solution.

**Operating conditions**—

Detector, column, column temperature, mobile phases A and B, and flow rate: Proceed as directed in the operating conditions in the Purity (2) under Mosapride Citrate Hydrate.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 40</td>
<td>85 – 45</td>
<td>15 – 55</td>
</tr>
</tbody>
</table>

Time span of measurement: For 40 minutes after sample injection, beginning after the solvent peak.

**System suitability**—

Test for required detectability: To exactly 1 mL of the standard solution add methanol to make exactly 25 mL. Confirm that the peak area of mosapride obtained with 10 µL of this solution is equivalent to 3.0 to 5.0% of that with 10 µL of the standard solution.

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mosapride are not less than 40,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mosapride is not more than 3.0%.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: the powder in single-unit container meets the requirement of the Content uniformity test.

To the total content of 1 container of Mosapride Citrate Powder add 5 mL of water, and shake. Then, add 20 mL of methanol, shake for 20 minutes, and add methanol to make exactly 50 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, add methanol to make exactly V/5 mL so that each mL contains about 20 µg of mosapride citrate (C₂₁H₂₅ClFN₃O₇.C₆H₈O₇), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of mosapride citrate (C₂₁H₂₅ClFN₃O₇.C₆H₈O₇) = Mₕ × A₁/Å₅ × V'/V × 1/50

Mₕ: Amount (mg) of mosapride citrate for assay, calculated on the anhydrous basis

**Dissolution** <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of the 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Mosapride Citrate Powder is not less than 70%.

Start the test with an amount of Mosapride Citrate Powder, equivalent to about 2.5 mg of mosapride citrate (C₂₁H₂₅ClFN₃O₇.C₆H₈O₇) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 µm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 30 mg of mosapride citrate for assay (separately determine the water <2.48> in the same manner as Mosapride Citrate Hydrate), and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 50 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01>, and determine the peak areas, A₁ and A₅, of mosapride of both solutions.

Dissolution rate (%) with respect to the labeled amount of mosapride citrate (C₂₁H₂₅ClFN₃O₇.C₆H₈O₇) = Mₕ/Mₜ × A₁/Å₅ × 1/C × 9

Mₜ: Amount (g) of sample
C: Labeled amount (mg) of mosapride citrate (C₂₁H₂₅ClFN₃O₇.C₆H₈O₇) in 1 g

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 8.82 g of trisodium citrate dihydrate in 800 mL of water, adjust to pH 3.3 with dilute hydrochloric acid, and add water to make 1000 mL. To 240 mL of this solution add 90 mL of methanol and 70 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of mosapride is about 9 minutes.

**System suitability**—

System performance: When the procedure is run with 50 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mosapride are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 µL of the standard solution under the above operat-
For the reference text, please refer to the original document.
Mosapride Citrate Tablets is not less than 80%.

Start the test with 1 tablet of Mosapride Citrate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 1 mL of the subsequent filtrate, add the dissolution medium to make exactly 1 mL, so that each mL contains about 2.8 μg of mosapride citrate (C₂₁H₂₅ClFN₃O₃.C₆H₈O₇) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of mosapride citrate for assay (separately, determine the water 2.48 in the same manner as Mosapride Citrate Hydrate), and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and determine the peak areas, A₁ and A₃, of mosapride of both solutions.

**Dissolution rate (%) with respect to the labeled amount of mosapride citrate (C₂₁H₂₅ClFN₃O₃.C₆H₈O₇) = \( \frac{M_S \times A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 9 \)\)

M₅: Amount (mg) of mosapride citrate for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of mosapride citrate (C₂₁H₂₅ClFN₃O₃.C₆H₈O₇) in 1 tablet

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 8.82 g of trisodium citrate dihydrate in 800 mL of water, adjust the pH to 3.3 with dilute hydrochloric acid, and add water to make 1000 mL. To 240 mL of this solution add 90 mL of methanol and 70 mL of hydrochloric acid, and add water to make 1000 mL. Dissolve in methanol to make exactly 100 mL. Pipet 2 mL of this solution as the sample solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and determine the peak areas, A₁ and A₃, of mosapride of both solutions.

Flow rate: Adjust the flow rate so that the retention time of mosapride is about 9 minutes.

**System suitability—**

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mosapride are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mosapride is not more than 2.0%.

**Assay**

Weigh accurately the mass of not less than 20 Mosapride Citrate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of mosapride citrate (C₂₁H₂₅ClFN₃O₃.C₆H₈O₇), and moisten with 2 mL of water. Add 70 mL of methanol, shake for 20 minutes, add methanol to make exactly 100 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 53 mg of mosapride citrate for assay (separately, determine the water 2.48 in the same manner as Mosapride Citrate Hydrate), and dissolve in methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry 2.24, and determine the absorbances, A₁ and A₃, at 273 nm.

Amount (mg) of mosapride citrate (C₂₁H₂₅ClFN₃O₃.C₆H₈O₇) = \( M_S \times A_T/A_S \times 1/5 \)

M₅: Amount (mg) of mosapride citrate for assay, calculated on the anhydrous basis

**Containers and storage**

Containers—Tight containers.

**Freeze-dried Live Attenuated Mumps Vaccine**

Freeze-dried Live Attenuated Mumps Vaccine is a dried preparation containing live attenuated mumps virus.

It conforms to the requirements of Freeze-dried Live Attenuated Mumps Vaccine in the Minimum Requirements of Biologic Products.

**Description**

Freeze-dried Live Attenuated Mumps Vaccine becomes a clear, colorless, yellowish or reddish liquid on addition of solvent.

**Mupirocin Calcium Hydrate**

Mupirocin Calcium Hydrate is the calcium salt of a substance having antibacterial activity produced by the growth of *Pseudomonas fluorescens*. It contains not less than 895 μg (potency) and not more than 970 μg (potency) per mg, calculated on the anhydrous basis. The potency of Mupirocin Calcium Hydrate is expressed as mass (potency) of mupirocin (C₅₂H₈₆CaO₁₈.2H₂O: 1075.34)

Monocalcium bis[9-((2E)-4-[[2S,3S,4R,5S]-2,3-epoxy-5-hydroxy-4-methylhexyl]-3,4-dihydroxy-3,4,5,6-tetrahydro-2H-pyran-2-yl]-3-methylbut-2-enoyloxy)nonanoate] dihydrate

[C₅₂H₈₆CaO₁₈.2H₂O: 1075.34]

Mupirocin Calcium Hydrate is freely soluble in methanol and slightly soluble in water and in ethanol (95%).

**Mupirocin Calcium Hydrate**
Identification (1) To 1 mL of a solution of Mupirocin Calcium Hydrate in methanol (1 in 200) add 4 mL of hydroxylamine perchlorate-ethanol TS and 1 mL of N,N'-dicyclohexylcarbodiimide-ethanol TS, shake well, and allow to stand in lukewarm water for 20 minutes. After cooling, add 1 mL of iron (III) perchlorate hexahydrate-ethanol TS to the solution, and shake; a dark purple color develops.

(2) Determine the absorption spectrum of a solution of Mupirocin Calcium Hydrate (in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 219 nm and 224 nm.

(3) Determine the infrared absorption spectrum of Mupirocin Calcium Hydrate as directed in thepaste method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1708 cm\(^{-1}\), 1648 cm\(^{-1}\), 1558 cm\(^{-1}\), 1321 cm\(^{-1}\), 1151 cm\(^{-1}\) and 894 cm\(^{-1}\).

(4) A solution of Mupirocin Calcium Hydrate (3 in 1000) corresponds to the Qualitative Tests <1.09> (3) for calcium salt.

Optical rotation <2.49> \([\alpha]_D^{20} = -16 - 20^\circ\) (1 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

Purity (1) Related substances—Dissolve 50 mg of Mupirocin Calcium Hydrate in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, and a solution of tetrahydrofuran (3 in 4) (1:1) to make 10 mL, and use this solution as the sample solution (1). Pipet 2 mL of the sample solution (1), add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, and a solution of tetrahydrofuran (3 in 4) (1:1) to make exactly 100 mL, and use this solution as the sample solution (2). Prepare these sample solutions at a temperature between 4°C and 8°C. Perform the test with exactly 20 μL of the sample solution (1) and the sample solution (2) as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the areas of each peak of the sample solution (1) and the sample solution (2) by the automatic integration method. Calculate the amount of the related substances by the following formula: the amount of principal related substance (appeared at about 0.7 of the relative retention time to mupirocin) is not more than 4.0%, and the total amount of related substances (the total area of the peaks other than of the solvent and mupirocin) is not more than 6.0%.

Amount (%) of principal related substance

\[
\text{Amount} = \frac{A_1}{A + A_n} \times 100 \times \frac{P \times 100}{100 - \frac{A \times 100}{A + A_n}}
\]

Total amount (%) of related substances

\[
\text{Total amount} = \frac{A}{A + A_n} \times 100 \times \frac{P \times 100}{100 - \frac{A \times 100}{A + A_n}}
\]

A: Total peak areas other than of the solvent and mupirocin from the sample solution (1)
A\(_1\): Peak area of the relative retention time of about 0.7 to mupirocin from the sample solution (1)
A\(_n\): A value of 50 times of peak area of mupirocin from the sample solution (2)
P: Potency per mg obtained from the assay

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of mupirocin beginning after the solvent peak.

System suitability—

Test for required detection: Pipet 1 mL of the sample solution (2), and add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, and a solution of tetrahydrofuran (3 in 4) (1:1) to make exactly 20 mL. Confirm that the peak area of mupirocin obtained from 20 μL of this solution is equivalent to 4 to 6% of that obtained from 20 μL of the sample solution (2).

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the sample solution (2) under the above operating conditions, the relative standard deviation of the peak areas of mupirocin is not more than 2.0%.

(2) Inorganic salt from manufacturing process—Being specified separately.

Water <2.48> Not less than 3.0% and not more than 4.5% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Mupirocin Calcium Hydrate and Mupirocin Lithium RS, equivalent to about 20 mg (potency), dissolve in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 and a solution of tetrahydrofuran (3 in 4) (1:1) to make exactly 200 mL, and use these solutions as the sample solution and the standard solution. Preserve these solutions at a temperature between 4°C and 8°C. Perform the test with exactly 20 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A\(_1\) and A\(_S\), of mupirocin of each solution.

Amount [μg (potency)] of mupirocin (C\(_{26}\)H\(_{44}\)O\(_9\))

\[
M_S = M_b \times A_1/A_S \times 1000
\]

M\(_S\): Amount [mg (potency)] of Mupirocin Lithium RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadsilsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 7.71 g of ammonium acetate in 750 mL of water, adjust the pH to 5.7 with acetic acid (100), and add water to make 1000 mL. To 300 mL of this solution add 100 mL of tetrahydrofuran.

Flow rate: Adjust the flow rate so that the retention time of mupirocin is about 12.5 minutes.

System suitability—

System performance: Dissolve about 20 mg of Mupirocin Lithium RS and about 5 mg of ethyl parahydroxybenzoate in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 and a solution of tetrahydrofuran (3 in 4) (1:1) to make 200 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, mupirocin and ethyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 12.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of mupirocin is not more than 1.0%.

Containers and storage  Containers—Tight containers.

**Mupirocin Calcium Ointment**

ミュピロシンカルシウム軟膏

Mupirocin Calcium Ointment is an oily ointment preparation.

Mupirocin Calcium Ointment contains not less than 95.0% and not more than 105.0% of the labeled potency of mupirocin (C_{26}H_{44}O_{9} \cdot 500.62).

Method of preparation  Prepare as directed under Ointments, with Mupirocin Calcium Hydrate.

Identification  To an amount of Mupirocin Calcium Ointment, equivalent to 10 mg (potency) of Mupirocin Calcium Hydrate according to the labeled amount, add 5 mL of water, and warm on a water bath at 60°C for 10 minutes while occasional shaking. After cooling, filter, and to 1 mL of the filtrate add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry 2.24: it exhibits a maximum between 220 nm and 224 nm.

Purity  Related substances—To an amount of Mupirocin Calcium Ointment, equivalent to 50 mg (potency) of Mupirocin Calcium Hydrate according to the labeled amount, add 5 mL of diluted tetrahydrofuran (3 in 4), and shake vigorously. Then, add 5 mL of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, shake vigorously, filter through a glass wool filter, and use the filtrate as the sample solution. Pipet 2 mL of the sample solution, add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, 10 mL of diluted tetrahydrofuran (3 in 4), and shake vigorously. Then, add exactly 10 mL of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, shake vigorously, filter through a glass wool filter, and use the filtrate as the sample solution. Pipet 2 mL of the sample solution, add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, and diluted tetrahydrofuran (3 in 4) (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.24 of the drug substance. Determine the amount of each related substance using the following equation: the amount of the related substance is not more than 4.0%, and the amount of each related substance is not more than 6.0%.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Mupirocin Calcium Hydrate.

Time span of measurement: About 5 times as long as the retention time of mupirocin, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 and diluted tetrahydrofuran (3 in 4) (1:1) to make exactly 20 mL. Confirm that the peak area of mupirocin obtained with 20 μL of this solution is equivalent to 4 to 6% of that with 20 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay under Mupirocin Calcium Hydrate.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mupirocin is not more than 2.0%.

Assay  Weigh accurately an amount of Mupirocin Calcium Ointment, equivalent to about 2 mg (potency) of Mupirocin Calcium Hydrate, add exactly 10 mL of diluted tetrahydrofuran (3 in 4), and shake vigorously. To this solution add exactly 10 mL of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, shake vigorously, filter through a glass wool filter, and use the filtrate as the sample solution. Separately, weigh accurately an amount of Mupirocin Lithium RS, equivalent to about 20 mg (potency), dissolve in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 and diluted tetrahydrofuran (3 in 4) (1:1) to make exactly 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Mupirocin Calcium Hydrate.

Amount [mg (potency)] of mupirocin (C_{26}H_{44}O_{9})

\[ M_5 = M_z \times \frac{A_5 - A_z \times 1/10}{A_z \times 10} \]

\( M_z \): Amount [mg (potency)] of Mupirocin Lithium RS

Containers and storage  Containers—Tight containers.

**Nabumetone**

ナブメトン

\[
\text{C}_{15}H_{16}O_2: \ 228.29 \\
\text{4-(6-Methoxynaphthalen-2-yl)butan-2-one} \ [42924-33-8]
\]

Nabumetone contains not less than 98.0% and not more than 101.0% of C_{15}H_{16}O_{2}, calculated on the anhydrous basis.

Description  Nabumetone occurs as white to yellowish white crystals or a crystalline powder.

It is soluble in acetonitrile, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.
Identification (1) Determine the absorption spectrum of a solution of Nabumetone in methanol (1 in 30,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nabumetone RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nabumetone as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Nabumetone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 79 – 84°C.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Nabumetone according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm). Proceed with 1.0 mg of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the peak area of the related substance G obtained from the sample solution is not larger than 3/5 times the peak area of nabumetone from the standard solution, and each peak area other than nabumetone and the related substance G is not larger than 1/5 times the peak area of nabumetone from the standard solution. Furthermore, the total area of the peaks other than nabumetone is not larger than 1.6 times the peak area of nabumetone from the standard solution. For these calculations, use each peak area of the related substances A, B, C, D, E, F and G, which are having the relative retention time of about 0.73, 0.85, 0.93, 1.2, 1.9, 2.6 and 2.7 with respect to nabumetone, after multiplying by their relative response factors, 0.12, 0.94, 0.25, 0.42, 1.02, 0.91 and 0.1, respectively.

Operating conditions—
Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase A: A mixture of water and acetic acid (100) (999:1).

Mobile phase B: A mixture of acetonitrile and tetrahydrofuran (7:3).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 12</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>12 – 28</td>
<td>60 → 20</td>
<td>40 → 80</td>
</tr>
</tbody>
</table>

Flow rate: 1.3 mL per minute.
Time span of measurement: About 3 times as long as the retention time of nabumetone, beginning after the solvent peak.

System suitability—
Test for required detectability: Pipet 2 mL of the standard solution, and add acetonitrile to make exactly 10 mL. Confirm that the peak area of nabumetone obtained from 10 μL of this solution is equivalent to 14 to 26% of that from 10 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nabumetone is not more than 5.0%.

Water <2.48> Not more than 0.2% (1 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 20 mg each of Nabumetone and Nabumetone RS (separately determine the water <2.48> in the same manner as Nabumetone), dissolve them in acetonitrile to make exactly 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak area of nabumetone, A1 and A5, from each solution.

Amount (mg) of C15H16O2 = M5 × A1/A5

M5: Amount (mg) of Nabumetone RS, calculated on the anhydrous basis.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 600 mL of a mixture of water and acetic acid (100) (999:1) add 400 mL of a mixture of acetonitrile and tetrahydrofuran (7:3).

Flow rate: Adjust the flow rate so that the retention time of nabumetone is about 10 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nabumetone are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nabumetone is not more than 1.0%.

Containers and storage Containers—Tight containers.
Nabumetone Tablets

ナブメトン錠

Nabumetone Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of nabumetone (C₁₅H₁₆O₂: 228.29).

Method of preparation Prepare as directed under Tablets, with Nabumetone.

Identification To a quantity of powdered Nabumetone Tablets, equivalent to 80 mg of Nabumetone according to the labeled amount, add 50 mL of methanol, shake for 10 minutes and centrifuge the solution. To 1 mL of the supernatant liquid, add methanol to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24> it exhibits maxima between 259 nm and 263 nm, between 268 nm and 272 nm, between 316 nm and 320 nm, and between 330 nm and 334 nm.

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Dissolution <6.10> When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of a solution of polysorbate 80 (dissolving 3 g of polysorbate 80 in water to make 100 mL) as the dissolution medium, the dissolution rate in 60 minutes of Nabumetone Tablets is not less than 70%.

Start the test with 1 tablet of Nabumetone Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add a solution, prepared by adding to 20 mL of ethanol (99.5) the dissolution medium to make 50 mL, to make exactly V mL so that each mL contains about 89 μg of nabumetone (C₁₅H₁₆O₂) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Nabumetone RS (separately determine the water <2.46> in the same manner as Nabumetone), and dissolve in ethanol (99.5) to make exactly 100 mL. Pipet 10 mL of this solution, add the dissolution medium to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances, A₁ and A₃, at 331 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared by adding to 20 mL of ethanol (99.5) the dissolution medium to make 50 mL as the blank.

Dissolution rate (%) with respect to the labeled amount of nabumetone (C₁₅H₁₆O₂)

\[ M_5 \times \frac{A_1}{A_3} \times \frac{V}{V} \times \frac{1}{C} \times \frac{360}{100} \]

M₅: Amount (mg) of Nabumetone RS, calculated on the anhydrous basis
C: Labeled amount (mg) of nabumetone (C₁₅H₁₆O₂) in 1 tablet

Assay Weigh accurately not less than 20 tablets of Nabumetone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.2 g of nabumetone (C₁₅H₁₆O₂), add 10 mL of water and shake, add 40 mL of methanol, shake for 30 minutes, and then add methanol to make exactly 100 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, then add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Nabumetone RS (separately determine the water <2.46> in the same manner as Nabumetone), dissolve by adding 50 mL of methanol and exactly 20 mL of the internal standard solution, then add methanol to make 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.61> according to the following conditions, and calculate the ratios, Q₁ and Q₃, of the peak area of nabumetone to that of the internal standard.

Amount (mg) of nabumetone (C₁₅H₁₆O₂)

\[ M_5 \times \frac{Q_1}{Q_3} \times 5 \]

M₅: Amount (mg) of Nabumetone RS, calculated on the anhydrous basis

Internal standard solution—Dissolve 0.12 g of 2-ethylhexyl parahydroxybenzoate in methanol to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile, water and acetic acid (100) (550:450:1).

Flow rate: Adjust the flow rate so that the retention time of nabumetone is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, nabumetone and the internal standard are eluted in this order with the resolution between these peaks being not less than 13.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nabumetone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.
Nadolol

Nadolol, when dried, contains not less than 98.0% of C₁₇H₂₇NO₄.

Description
Nadolol occurs as a white to yellow-brownish white crystalline powder.
It is freely soluble in methanol and in acetic acid (100), soluble in ethanol (95), and slightly soluble in water and in chloroform.
A solution of Nadolol in methanol (1 in 100) shows no optical rotation.
Melting point: about 137°C.

Identification
(1) Determine the absorption spectrum of a solution of Nadolol in methanol (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.
(2) Determine the infrared absorption spectrum of Nadolol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, so that its transmittance at an absorption band at a wave number of about 1585 cm⁻¹ is 25 to 30%, and determine the infrared absorption spectrum between 1600 cm⁻¹ and 1100 cm⁻¹. Determine the absorbances, A₁₂₅₀ and A₁₂₆₅, from the transmittances, T₁₂₅₀ and T₁₂₆₅, at wave numbers of about 1265 cm⁻¹ (racemic substance A) and 1250 cm⁻¹ (racemic substance B), respectively: the ratio A₁₂₆₅/A₁₂₅₀ is between 0.72 and 1.08.

Assay
Weigh accurately about 0.28 g of Nadolol, previously dried, dissolve in 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to green-blue (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 30.94 mg of C₁₇H₂₇NO₄

Containers and storage
Containers—Tight containers.
Storage—Light-resistant.
Nafamostat Mesilate

ナファモスタットメシル酸塩

C₁₉H₁₇N₅O₂·2CH₄O₃S: 539.58
6-Amidinonaphthalen-2-yl 4-guanidinobenzoate bis(methanesulfonate)
[82956-11-4]

Nafamostat Mesilate, when dried, contains not less than 99.0% and not more than 101.0% of C₁₉H₁₇N₅O₂·2CH₄O₃S.

Description Nafamostat Mesilate occurs as a white crystalline powder.

It is freely soluble in formic acid, soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in 0.01 mol/L hydrochloric acid TS.

Melting point: about 262°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Nafamostat Mesilate in 0.01 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.44>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nafamostat Mesilate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A 0.1-g portion of Nafamostat Mesilate responds to the Qualitative Tests <1.09> (1) for mesilate.

pH <2.54> The pH of a solution prepared by dissolving 1.0 g of Nafamostat Mesilate in 50 mL of water is between 4.7 and 5.7.

Purity (1) Clarity and color of solution—A solution prepared by dissolving 1.0 g of Nafamostat Mesilate in 50 mL of water is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Nafamostat Mesilate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 0.10 g of Nafamostat Mesilate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 10 mL of the sample solution, add the mobile phase to make exactly 100 mL. Then pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of each solution by the automatic integration method: the area of each peak other than nafamostat obtained from the sample solution is not larger than 1/5 times the peak area of nafamostat from the standard solution. Furthermore, the total area of the peaks other than nafamostat is not larger than the peak area of nafamostat from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 6.07 g of sodium 1-heptane sulfonate in 1000 mL of diluted acetic acid (100) (3 in 500). To 700 mL of this solution add 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nafamostat is about 7 minutes.

Time span of measurement: About 4 times as long as the retention time of nafamostat, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Pipet 15 mL of this solution, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of nafamostat obtained from 10 µL of this solution is equivalent to 1.1 to 1.9% of that from 10 µL of the standard solution.

System performance: Dissolve 0.1 g of nafamostat mesilate in the mobile phase to make 100 mL. To 10 mL of this solution add the mobile phase to make 100 mL. To 5 mL of this solution add 5 mL of a solution of 6-amidino-2-naphthol methanesulfonate in the mobile phase (1 in 20,000). When the procedure is run with 10 µL of this solution under the above operating conditions, 6-amidino-2-naphthol and nafamostat are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nafamostat is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Nafamostat Mesilate, previously dried, dissolve in 4 mL of formic acid, add 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 26.98 mg of C₁₉H₁₇N₅O₂·2CH₄O₃S

Containers and storage Containers—Tight containers.
Nalidixic Acid

Nalidixic acid is not larger than 2.5 times the peak area of nalidixic acid with the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 260 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 6.24 g of sodium dihydrogen phosphate dihydrate in 950 mL of water, adjust the pH to 2.8 with phosphoric acid, and add water to make 1000 mL. To 300 mL of this solution add 200 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of nalidixic acid is about 19 minutes.
Time span of measurement: About 3 times as long as the retention time of nalidixic acid beginning after the solvent peak.

System suitability—
Test for required detectability: Pipet 5 mL of the standard solution, and add water to make exactly 10 mL. Confirm that the peak area of nalidixic acid obtained with 10 μL of this solution is equivalent to 40 to 60 μL of that with 10 μL of the standard solution.
System performance: Dissolve 25 mg of methyl parahydroxybenzoate in 100 mL of a mixture of water and methanol (1:1). To 1 mL of this solution add water to make 10 mL. To 5 mL of this solution add 5 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, methyl parahydroxybenzoate and nalidixic acid are eluted in the order with the resolution between these peaks being not less than 13.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nalidixic acid is not more than 2.0%.

Loss on drying <2.41> Not more than 0.20% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.3 g of Nalidixic Acid, previously dried, dissolve in 50 mL of N,N-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethyl ammonium hydroxide VS (potentiometric titration). Separately, to 50 mL of N,N-dimethylformamide add 13 mL of a mixture of water and methanol (89:11), perform a blank determination with the solution, and make any necessary correction.
Each mL of 0.1 mol/L tetramethyl ammonium hydroxide VS = 23.22 mg of C₁₂H₁₂N₂O₃.

Containers and storage Containers—Tight containers.
Naloxone Hydrochloride

Naloxone Hydrochloride contains not less than 98.5% of C<sub>19</sub>H<sub>21</sub>NO<sub>4</sub>·HCl, calculated on the dried basis.

Description
Naloxone Hydrochloride occurs as white to yellowish white, crystals or crystalline powder. It is freely soluble in water, soluble in methanol, slightly soluble in ethanol (99.5) and in acetic acid (100), and very slightly soluble in acetic anhydride.

It is hygroscopic. It is gradually colored by light.

Identification (1)
Determine the absorption spectrum of a solution of Naloxone Hydrochloride (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Naloxone Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Naloxone Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49> [α]<sub>D</sub><sup>23</sup> = -170 – -181° (0.25 g calculated on the dried basis, water, 10 mL, 100 mm).

pH <2.54> Dissolve 0.10 g of Naloxone Hydrochloride in 10 mL of freshly boiled and cooled water: the pH of the solution is between 4.5 and 5.5.

Purity
Related substances—Conduct this procedure as rapidly as possible without exposure to light, using light-resistant containers. Dissolve 0.08 g of Naloxone Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.67>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of ammonia-saturated 1-butanol TS and methanol (20:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly iron (III) chloride-potassium hexacyano-ferrate (III) TS on the plate: the number of the spot other than the principal spot from the sample solution is not more than 1 and it is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 2.0% [0.1 g, 105°C, 5 hours. Use a desiccator (phosphorus (V) oxide) for cooling].

Residue on ignition <2.44> Not more than 0.2% (0.1 g).

Assay
Weigh accurately about 0.3 g of Naloxone Hydrochloride, dissolve in 80 mL of acetic acid (100) by warming. After cooling, add 80 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 36.38 mg of C<sub>19</sub>H<sub>21</sub>NO<sub>4</sub>·HCl

Containers and storage
Containers—Tight containers.
Storage—Light-resistant.

Naphazoline Hydrochloride

Naphazoline Hydrochloride, when dried, contains not less than 98.5% of C<sub>14</sub>H<sub>14</sub>N<sub>2</sub>·HCl.

Description
Naphazoline Hydrochloride occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water, soluble in ethanol (95) and in acetic acid (100), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Melting point: 255 – 260°C (with decomposition).

Identification (1) To 10 mL of a solution of Naphazoline Hydrochloride (1 in 100) add 5 mL of bromine TS, and boil: a deep purple color develops.

(2) To 30 mL of a solution of Naphazoline Hydrochloride (1 in 100) add 2 mL of sodium hydroxide TS, and extract with two 25-mL portions of diethyl ether. Evaporate the combined diethyl ether extracts to dryness with the aid of a current of air. Dry the residue at 80°C for 1 hour: the residue melts <2.66> between 117°C and 120°C.

(3) Dissolve 0.02 g of the residue obtained in (2) in 2 to 3 drops of dilute hydrochloric acid and 5 mL of water, and add 2 mL of Reinecke salt TS: a red-purple, crystalline precipitate is formed.

(4) A solution of Naphazoline Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 0.10 g of Naphazoline Hydrochloride...
Naphazoline Nitrate

Naphazoline Nitrate occurs as a white, crystalline powder. It is odorless, and has a bitter taste. It is freely soluble in acetic acid (100), soluble in ethanol (95), sparingly soluble in water, slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Identification (1) To 10 mL of a solution of Naphazoline Nitrate (1 in 100) add 5 mL of sodium hydroxide TS, and extract with two 25-mL portions of diethyl ether. Combine the diethyl ether extracts, evaporate to dryness with the aid of a current of air, and dry the residue at 80°C, 2 mL of sodium hydroxide TS and 1 mL of a solution of copper (II) chloride dihydrate in ethanol (95) (1 in 10), and shake: a blue color is produced (chlorobutanol).

(2) A solution of Naphazoline Nitrate (1 in 20) responds for nitrate.

pH <2.50 Dissolve 0.1 g of Naphazoline Nitrate in 10 mL of freshly boiled and cooled water: the pH of the solution is between 5.0 and 7.0.

Melting point <2.60 167 – 170°C.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Naphazoline Nitrate in 50 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07—Proceed with 1.0 g of Naphazoline Nitrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.40 Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44 Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Naphazoline Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 24.67 mg of C₁₄H₁₄N₂.HCl

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Naphazoline and Chlorpheniramine Solution

Naphazoline and Chlorpheniramine Solution contains not less than 0.045 w/v% of naphazoline nitrate (C₁₄H₁₄N₂.HNO₃: 273.29), and not less than 0.09 w/v% and not more than 0.11 w/v% of chlorpheniramine maleate (C₁₈H₁₉ClN₂.C₂H₄O₄: 390.86).

Method of preparation

| Chloropheniramine Maleate | 1 g |
| Glycerin | 2 g |
| Purified Water or Purified | 50 mL |

To make 1000 mL

Dissolve, and mix the above ingredients.

Description Naphazoline and Chlorpheniramine Solution is a clear, colorless liquid.

Identification (1) To 20 mL of Naphazoline and Chlorpheniramine Solution add 2 mL of a solution of potassium hydroxide (7 in 10) and 5 mL of pyridine, and heat at 100°C for 5 minutes: a red color is produced (chlorobutanol).

(2) Place 10 mL of Naphazoline and Chlorpheniramine Solution in a glass-stoppered test tube, add 10 mL of ethanol (95), 2 mL of sodium hydroxide TS and 1 mL of a solution of copper (II) chloride dihydrate in ethanol (95) (1 in 10), and shake: a blue color is produced (glycerin).
(3) To 20 mL of Naphazoline and Chlorpheniramine Solution add 5 mL of sodium hydroxide TS, extract with 10 mL of diethyl ether, and separate the diethyl ether layer. Take 5 mL of this solution, distill off the solvent, dissolve the residue in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 0.01 g each of naphazoline nitrate and Chlorpheniramine Maleate RS in 10 mL and 5 mL of methanol, respectively, and use these solutions as standard solutions (1) and (2). Perform the test with these solutions as directed under Thin-layer Chromatography 5.07. Spot 5 μL each of the sample solution and standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, acetone and ammonia solution (28) (73:15:10:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): two spots from the sample solution exhibit the same Rf values as the spots from standard solutions (1) and (2). Spray evenly Dragendorff’s TS on the plate: the spots from standard solutions (1) and (2) and the corresponding spot from the sample solutions reveal an orange color.

**Assay** Pipet 4 mL of Naphazoline and Chlorpheniramine Solution, add exactly 4 mL of the internal standard solution, then add water to make 10 mL, and use this solution as the sample solution. Weigh accurately about 50 mg of naphazoline nitrate for assay, dried at 105 °C for 2 hours, and about 0.1 g of Chlorpheniramine Maleate RS, dried at 105 °C for 3 hours, dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add exactly 4 mL of the internal standard solution, then add water to make 10 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solutions as directed under Liquid Chromatography 5.07 according to the following conditions, and calculate the ratios, Q_{Ta} and Q_{Tb}, of the peak height of naphazoline and chlorpheniramine to that of the internal standard of the sample solution, and the ratios, Q_{Sa} and Q_{Sb}, of the peak height of naphazoline and chlorpheniramine to that of the internal standard of the standard solution.

\[
\begin{align*}
\text{Amount (mg) of naphazoline nitrate (C_{14}H_{14}N_2.HNO_3)} &= M_{Sa} \times \frac{Q_{Ta}}{Q_{Sb}} \times \frac{1}{25} \\
\text{Amount (mg) of chlorpheniramine maleate (C_{16}H_{19}ClN_2.C_4H_4O_4)} &= M_{Sb} \times \frac{Q_{Tb}}{Q_{Sb}} \times \frac{1}{25} \\
M_{Sa}: \text{Amount (mg) of naphazoline nitrate for assay} \\
M_{Sb}: \text{Amount (mg) of Chlorpheniramine Maleate RS}
\end{align*}
\]

**Internal standard solution**—A solution of ethenazamide in methanol (1 in 1000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in inside diameter and 25 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of acetonitrile and a solution of sodium laurylsulfate (1 in 500) in diluted phosphoric acid (1 in 1000) (1:1).

Flow rate: Adjust the flow rate so that the retention time of chlorpheniramine is about 10 minutes.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions. Use a column giving well-resolved peaks of the internal standard, naphazoline and chlorpheniramine in this order.

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

**Naproxen**

![Naproxen structure](image)

C_{13}H_{16}O_{2}: 230.26

(2S)-2-(6-Methoxynaphthalen-2-yl)propanoic acid [22204-53-1]

Naproxen, when dried, contains not less than 98.5% of C_{13}H_{16}O_{2}.

**Description** Naproxen occurs as white crystals or crystalline powder. It is odorless.

It is freely soluble in acetone, soluble in methanol, in ethanol (99.5) and in chloroform, sparingly soluble in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

**Identification** (1) Dissolve 0.01 g of Naproxen in 5 mL of methanol, add 5 mL of water, then add 2 mL of potassium iodide TS and 5 mL of a solution of potassium iodate (1 in 100), and shake: a yellow to yellow-brown color develops. To this solution add 5 mL of chloroform, and shake: a light red-purple color develops in the chloroform layer.

(2) To 1 mL of a solution of Naproxen in ethanol (99.5) (1 in 300) add 4 mL of hydroxyamine perchlorate-dehydrated ethanol TS and 1 mL of N,N'-dicyclohexylcarbodiimide-dehydrated ethanol TS, shake well, and allow to stand in lukewarm water for 20 minutes. After cooling, add 1 mL of iron (III) perchlorate-dehydrated ethanol TS, and shake: a red-purple color develops.

(3) Determine the absorption spectrum of a solution of Naproxen in ethanol (99.5) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry 3.24D, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Naproxen, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry 3.24D, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** [α]_D^25: +63.0° to +68.5° (after drying, 0.1 g, chloroform, 10 mL, 100 mm).

**Melting point** 2.60D 154 – 158°C.

**Purity** (1) Clarity of solution—Dissolve 2.0 g of Naproxen in 20 mL of acetone: the solution is clear. Perform the
test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24> : the absorbance at 400 nm is not more than 0.070.

(2) Heavy metals <1.07> — Proceed with 2.0 g of Naproxen according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11> — Prepare the test solution with 2.0 g of Naproxen according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substances — Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Naproxen in 10 mL of a mixture of chloroform and ethanol (99.5) (1:1), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add a mixture of chloroform and ethanol (99.5) (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add a mixture of chloroform and ethanol (99.5) (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, dichloromethane, tetrahydrofuran and acetic acid (100) (50:30:17:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm); the spots other than the principal spot and the spot of the starting point from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> — Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> — Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Naproxen, previously dried, add 100 mL of dilute methanol (4 in 5), dissolve by gentle warming if necessary, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 23.03 mg of C19H27NO3.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

## Nateglinide

ナテグリニド

\[
\text{C}_{10}\text{H}_{15}\text{NO}_3; \quad 317.42
\]

\(N\)-(trans-4-(1-Methylethyl)cyclohexanecarbonyl)-D-phenylalanine [103816-04-4]

Nateglinide, when dried, contains not less than 98.0% and not more than 102.0% of C10H15NO3.

**Description** Nateglinide occurs as a white crystalline powder.

It is freely soluble in methanol and in ethanol (99.5), sparingly soluble in acetonitrile, and practically insoluble in water.

It dissolves in dilute sodium hydroxide TS.

**Identification** (1) Determine the absorption spectrum of a solution of Nateglinide in methanol (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nateglinide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nateglinide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Nateglinide RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample and the reference standard according to the method otherwise specified, filter and dry the crystals, and perform the test with the crystals.

**Optical rotation** <2.49> \([\alpha]_D^{20} \approx -36.5 - -40.0^\circ\) (after drying 0.2 g, dilute sodium hydroxide TS, 20 mL, 100 mm).

**Purity** (1) Heavy metals <1.07> — Proceed with 2.0 g of Nateglinide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances — Dissolve 0.25 g of Nateglinide in 20 mL of acetonitrile. To 4 mL of this solution add the mobile phase to make 25 mL, and use this solution as the sample solution. Pipet 2.5 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than nateglinide from the sample solution is not larger than the peak area of nateglinide from the standard solution.

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**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nateglinide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Nateglinide Tablets

Nateglinide Tablets contain not less than 96.0% and not more than 104.0% of the labeled amount of nateglinide (C_{19}H_{27}NO_{3}: 317.42).

Method of preparation Prepare as directed under Tablets, with Nateglinide.

Identification To an amount of powdered Nateglinide Tablets, equivalent to 20 mg of Nateglinide according to the labeled amount, add 20 mL of methanol, shake, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24> it exhibits maxima between 246 nm and 250 nm, between 251 nm and 255 nm, between 257 nm and 261 nm and between 262 nm and 266 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Nateglinide Tablets add 10 mL of 0.05 mol/L sodium dihydrogen phosphate TS adjusted to pH 2.5 with phosphoric acid, shake to disintegrate the tablet, and disperse to fine particles with the aid of ultrasonic waves. Add exactly 3V/50 mL of the internal standard solution, add 3V/5 mL of acetonitrile, shake for 10 minutes, and add acetonitrile to make V mL so that each mL contains about 0.6 mg of nateglinide (C_{19}H_{27}NO_{3}). Filter the solution through a membrane filter with a pore size not exceeding 0.45 μm, and discard the first 5 mL of the filtrate. To 8 mL of the subsequent filtrate add the mobile phase to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Nateglinide RS, previously dried at 105°C for 2 hours, and dissolve in acetonitrile to make exactly 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_1 and Q_8, of the peak area of nateglinide to that of the internal standard.

\[
M_5: \text{Amount (mg) of Nateglinide RS}
\]

Internal standard solution—A solution of propyl parahydroxybenzoate in acetonitrile (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust 0.05 mol/L sodium dihydrogen phosphate TS to pH 2.5 with phosphoric acid. To 550 mL of this solution add 450 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of nateglinide is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and nateglinide are eluted in this order with the resolution between these peaks being not less than 19.


**System suitability**

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and nateglinide are eluted in this order with the resolution between these peaks being not less than 19.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nateglinide to that of the internal standard is not more than 1.0%.

**Dissolution 6.10**

When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of the 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of a 30-mg tablet and that in 30 minutes of a 90-mg tablet of Nateglinide Tablets is not less than 75%, respectively.

Start the test with 1 tablet of Nateglinide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add the dissolution medium to make exactly V mL so that each mL contains about 33 μg of nateglinide (C<sub>19</sub>H<sub>27</sub>NO<sub>3</sub>) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 6 mg of nateglinide (C<sub>19</sub>H<sub>27</sub>NO<sub>3</sub>). Filter this solution with a pore size not exceeding 0.45 μm, discard the first 5 mL of the filtrate, to 4 mL of the subsequent filtrate add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of Nateglinide RS, previously dried at 105°C for 2 hours, add exactly 1 mL of the internal standard solution, and add acetonitrile to make 10 mL. To 4 mL of this solution add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the ratios, Q<sub>T</sub> and Q<sub>S</sub>, of the peak area of nateglinide to that of the internal standard.

\[
M_S = \frac{\text{Amount (mg) of Nateglinide RS}}{C} \times \frac{Q_T}{Q_S} \times \frac{V}{200}
\]

\[M_S: \text{Amount (mg) of Nateglinide RS}
\]

**Internal standard solution**—A solution of propyl parahydroxybenzoate in acetonitrile (3 in 125).

**Operating conditions**—

- Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 40°C.
- Mobile phase: Adjust to pH 2.5 of 0.05 mol/L sodium dihydrogen phosphate TS with phosphoric acid. To 550 mL of this solution add 450 mL of acetonitrile.
- Flow rate: Adjust the flow rate so that the retention time of nateglinide is about 10 minutes.

**System suitability**—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and nateglinide are eluted in this order with the resolution between these peaks being not less than 19.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nateglinide to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.
Neostigmine Methylsulfate

**Identification**

(1) Determine the absorption spectrum of a solution of Neostigmine Methylsulfate (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2,24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Neostigmine Methylsulfate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Neostigmine Methylsulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2,25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Neostigmine Methylsulfate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**pH** <2,24> Dissolve 1.0 g of Neostigmine Methylsulfate in 10 mL of freshly boiled and cooled water: the pH of the solution is between 3.0 and 5.0.

**Melting point** <2,60> 145 – 149°C.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Neostigmine Methylsulfate in 10 mL of water: the solution is clear and colorless.

(2) Sulfate—Dissolve 0.20 g of Neostigmine Methylsulfate in 10 mL of water, add 1 mL of dilute hydrochloric acid and 1 mL of barium chloride TS: no turbidity is produced immediately.

(3) Dimethylinophenol—Dissolve 0.10 g of Neostigmine Methylsulfate in 5 mL of water, and while cooling with ice, add 1 mL of diazoobenzensulfonic acid TS: no color develops.

**Loss on drying** <2,41> Not more than 1.0% (1 g, 105°C, 3 hours).

**Residue on ignition** <2,44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 25 mg each of Neostigmine Methylsulfate and Neostigmine Methylsulfate RS, previously dried, dissolve each in the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2,08> according to the following conditions, and determine the peak areas, A_T and A_S, of neostigmine in each solution.

\[
M_T = \text{Amount (mg) of C}_{13}H_{22}N_2O_6S = m_T \times A_T/A_S
\]

\[
M_S = \text{Amount (mg) of Neostigmine Methylsulfate RS}
\]

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 259 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3.12 g of sodium dihydrogenphosphate dihydrate in 1000 mL of water, adjust to pH 3.0 with phosphoric acid, and add 0.871 g of sodium 1-pentanesulfonate to dissolve. To 890 mL of this solution add 110 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of neostigmine is about 9 minutes.

**System suitability**—

System performance: Dissolve 25 mg of Neostigmine Methylsulfate and 4 mg of dimethylinophenol in 50 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, dimethylinophenol and neostigmine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of neostigmine methylsulfate is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.

**Neostigmine Methylsulfate Injection**

ネオスチグミンメチル硫酸塩注射液

Neostigmine Methylsulfate Injection is an aqueous solution for injection.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of neostigmine methylsulfate (C_{13}H_{22}N_2O_6S: 334.39).

**Method of preparation** Prepare as directed under Injections, with Neostigmine Methylsulfate.

**Description** Neostigmine Methylsulfate Injection is a clear, colorless liquid.

It is slowly affected by light.

pH: 5.0 – 6.5

**Identification** Take a volume of Neostigmine Methylsulfate Injection equivalent to 5 mg of neostigmine methylsulfate according to the labeled amount, add water to make 10 mL if necessary, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectro-
photometry <2.24> it exhibits a maximum between 257 nm and 261 nm.

Bacterial endotoxins <4.01> Less than 5 EU/mg.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Use Neostigmine Methylsulfate Injection as the sample solution. Separately, weigh accurately about 25 mg of Neostigmine Methylsulfate RS, previously dried at 105°C for 3 hours, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Neostigmine Methylsulfate.

Amount (mg) of neostigmine methylsulfate (C_{13}H_{22}N_{2}O_{6}S) = M_S \times A_T/A_S

M_S: Amount (mg) of Neostigmine Methylsulfate RS

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

Nicardipine Hydrochloride

ニカルジピン塩酸塩

\[
\text{C}_{26}\text{H}_{29}\text{N}_{3}\text{O}_{6}\text{HCl}: 515.99
\]

2-[Benzyl(methyl)amino]ethyl methyl (4RS)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate monohydrochloride

[34527-84-3]

Nicardipine hydrochloride, when dried, contains not less than 98.5% of C_{26}H_{29}N_{3}O_{6}HCl.

Description Nicardipine Hydrochloride occurs as a pale greenish yellow crystalline powder.

It is freely soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (99.5), and slightly soluble in water, in acetonitrile and in acetic anhydride.

A solution of Nicardipine Hydrochloride in methanol (1 in 20) shows no optical rotation.

It is gradually affected by light.

Identification (1) Determine the absorption spectrum of a solution of Nicardipine Hydrochloride in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nicardipine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.02 g of Nicardipine Hydrochloride in 10 mL of water and 3 mL of nitric acid: the solution responds to the Qualitative Tests <1.06> for chloride.

Melting point <2.60> 167 – 171°C.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Nicardipine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Nicardipine Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, then take exactly 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of nicardipine from the sample solution is not larger than the peak area of nicardipine from the standard solution, and the total area of each peak other than the peak of nicardipine is not larger than 2 times the peak area of nicardipine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of a solution of perchloric acid (43 in 50,000) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of nicardipine is about 6 minutes.

Time span of measurement: About 4 times as long as the retention time of nicardipine beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of nicardipine obtained from 10 µL of this solution is equivalent to 8 to 12% of that from 10 µL of the standard solution.

System performance: Dissolve 2 mg each of Nicardipine Hydrochloride and nifedipine in 50 mL of the mobile phase. When the procedure is run with 10 µL of this solution under the above operating conditions, nicardipine and nifedipine are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of nicardipine is not more than 3%.
Loss on drying <2.4> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately about 0.9 g of Nicardipine Hydrochloride, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 51.60 mg of \( \text{C}_{26}\text{H}_{29}\text{N}_{3}\text{O}_{6}\cdot\text{HCl} \)

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Nicardipine Hydrochloride Injection

### Description
Nicardipine Hydrochloride Injection occurs as a clear pale yellow liquid.

It is gradually changed by light.

### Identification
To a volume of Nicardipine Hydrochloride Injection, equivalent to 1 mg of Nicardipine Hydrochloride according to the labeled amount, add ethanol (99.5) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 235 nm and 239 nm, and between 351 nm and 355 nm.

pH <2.54> 3.0 – 4.5

Purity Related substances—Conduct the procedure without exposure to daylight using light-resistant vessels. To a volume of Nicardipine Hydrochloride Injection, equivalent to 5 mg of Nicardipine Hydrochloride according to the labeled amount, add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. To exactly 2 mL of the sample solution add the mobile phase to make exactly 20 mL. Determine the absorption spectrum of this solution according to the labeled amount, add ethanol (99.5) to make 100 mL. Determine the absorption spectrum of this solution and methanol to make 50 mL, and use this solution as the sample solution.

Perform the test with exactly 10 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the areas of the peaks other than nicardipine from the sample solution and the standard solution, and the total area of the peaks other than nicardipine is not larger than 2 times of the peak area of nicardipine from the standard solution.

\[ S = M_5 \times Q_s \times Q_e \times 1/25 \]

**M_5**: Amount (mg) of nicardipine hydrochloride for assay

**Internal standard solution**—A solution of di-n-butyl phthalate in methanol (1 in 625).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanilized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).
Column temperature: A constant temperature of about 40°C.

System suitability—Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of nicardipine obtained from 10 \( \mu \)L of this solution is equivalent to 8 to 12% of that from 10 \( \mu \)L of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 5 times with 10 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of nicardipine is not more than 1.0%.

Bacterial endotoxins <4.01> Less than 8.33 EU/mg.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.
Nicergoline / Official Monographs

Nicergoline

**Description** Nicergoline occurs as white to light yellow, crystals or crystalline powder.

It is soluble in acetonitrile, in ethanol (99.5) and in acetic anhydride, and practically insoluble in water.

It is gradually colored to light brown by light.

Melting point: about 136°C (with decomposition).

**Identification (1)** Determine the absorption spectrum of a solution of Nicergoline in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2,24>., and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nicergoline as directed in the potassium bromide disk method under Infrared Spectrophotometry <2,25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2,49> \([\alpha]_D^{20} + 5.2 - +6.2^\circ\) (after drying, 0.5 g, ethanol (95), 10 mL, 100 mm).

**Purity (1)** Heavy metals <1.07>—Proceed with 2.0 g of Nicergoline according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 25 mg of Nicergoline in 25 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetonitrile to make exactly 100 mL. Pipet 10 mL of this solution, add acetonitrile to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 mL each of the sample solution and standard solution as directed under Liquid Chromatography <2,01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.5 with respect to nicergoline, is not larger than 4 times the peak area of nicergoline from the standard solution, and the area of the peak other than nicergoline and other than the peak mentioned above is not larger than 2.5 times the peak area of nicergoline from the standard solution. The peak which area is larger than the peak area of nicergoline from the standard solution is not more than two peaks, and the total area of the peaks other than the peak of nicergoline is not larger than 7.5 times the peak area of nicergoline from the standard solution.

**Operating conditions**

- **Detector:** An ultraviolet absorption photometer (wavelength: 288 nm).
- **Column:** A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- **Column temperature:** A constant temperature of about 25°C.
- **Mobile phase:** Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.0 with triethylamine. To 350 mL of this solution add 350 mL of methanol and 300 mL of acetonitrile.
- **Flow rate:** Adjust the flow rate so that the retention time of nicergoline is about 25 minutes.
- **Time span of measurement:** About 2 times as long as the retention time of nicergoline beginning after the solvent peak.

**System suitability**

- **Test for required detectability:** To 1 mL of the sample solution add acetonitrile to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add acetonitrile to make exactly 100 mL. Confirm that the peak area of nicergoline obtained with 20 μL of this solution is equivalent to 3 to 7% of that with 20 μL of the solution for system suitability test.

**System performance:** When the procedure is run with 20 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nicergoline are not less than 8000 and not more than 2.0, respectively.

**System repeatability:** When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 4.0%.

**Loss on drying** <2,47> Not more than 0.5% (2 g, in vacuum, 60°C, 2 hours).

**Residue on ignition** <2,44> Not more than 0.1% (1 g).
Assay Weigh accurately about 0.4 g of Nicergoline, previously dried, add 10 mL of acetic anhydride, and warm to dissolve. After cooling, add 40 mL of nitrobenzene, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes to blue-green from red through a blue-purple (indicator: 10 drops of neutral red TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 24.22 mg of C$_{24}$H$_{26}$BrN$_3$O$_3$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Nicergoline Powder

ニセルゴリン散

Nicergoline Powder contains not less than 95.0% and not more than 105.0% of the labeled amount of nicergoline (C$_{24}$H$_{26}$BrN$_3$O$_3$: 484.39).

Method of preparation Prepare as directed under Granules or Powders, with Nicergoline.

Identification Vigorously shake for 10 minutes a quantity of Nicergoline Powder, equivalent to 10 mg of Nicergoline according to the labeled amount, with 20 mL of diluted ethanol (4 in 5), and centrifuge for 10 minutes. To 2 mL of the supernatant liquid add ethanol (99.5) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>:

\[
\text{Area} = \frac{S}{T} \times \left( \frac{A_{T_1}}{A_{T_2}} - \frac{A_{S_1}}{A_{S_2}} \right) \times 1/C \times 9
\]

where:

- \( S \) = Amount (mg) of nicergoline for assay,
- \( T \) = Amount (g) of sample,
- \( C \) = Labeled amount (mg) of nicergoline (C$_{24}$H$_{26}$BrN$_3$O$_3$) in 1 g.

Dissolution</ref> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 15 minutes of Nicergoline Powder is not less than 80%.

Start the test with an accurately weighed amount of Nicergoline Powder, equivalent to about 5 mg of nicergoline (C$_{24}$H$_{26}$BrN$_3$O$_3$) according to the labeled amount, withdraw not less than about 20 mL of the medium at the specified minute after starting the test, and filter through a laminated polyester fiber. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of nicergoline for assay, previously dried in vacuum at 60°C for 2 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, and add the dissolution medium to make exactly 100 mL. Pipet 10 mL of this solution, and add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances at 225 nm, \( A_{T_1} \), and \( A_{S_1} \), and at 250 nm, \( A_{T_2} \) and \( A_{S_2} \), of the sample solution and standard solution as directed under Liquid Chromatography <2.24>, using the dissolution medium as the blank.

\[
\text{Dissolution rate} = \frac{\text{Sample} - \text{Blank}}{100} \times 100%
\]

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 288 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μm in particle diameter).

Temperature: A constant temperature of about
Nicergoline Tablets

40°C. Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.0 with triethylamine. To 350 mL of this solution add 350 mL of methanol and 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nicergoline is about 25 minutes.

System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nicergoline are not less than 8000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 1.0%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Nicergoline Tablets

ニセルゴリン錠

Nicergoline Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of nicergoline (C24H26BrN3O3; 484.39).

Method of preparation Prepare as directed under Tablets, with Nicergoline.

Identification Take a quantity of powdered Nicergoline Tablets, equivalent to 10 mg of Nicergoline according to the labeled amount, add 20 mL of ethanol (99.5), shake vigorously for 10 minutes, and filter through a 0.45-μm pore-size membrane filter. To 2 mL of the filtrate add ethanol (99.5) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits maxima between 226 nm and 230 nm, and between 286 nm and 290 nm.

Purity Related substances—Perform the test with 20 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of substances other than nicergoline by the area percentage method: the total amount of them is not more than 2.0%.

Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of nicergoline beginning after the solvent peak.

System suitability—
Test for required detectability: To 1 mL of the standard solution obtained in the Assay add a mixture of acetonitrile and water (17:3) to make 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add the mixture of acetonitrile and water (17:3) to make exactly 100 mL. Confirm that the peak area of nicergoline obtained with 20 μL of this solution is equivalent to 3 to 7% of that with 20 μL of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 1.5%.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Nicergoline Tablets add exactly 25 mL of diluted ethanol (4 in 5), disperse to fine particles with the aid of ultrasonic wave, and shake for 5 minutes. Centrifuge this solution for 10 minutes, pipet exactly 4 mL of the supernatant liquid, add diluted ethanol (4 in 5) to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of nicergoline for assay, previously dried in vacuum at 60°C for 2 hours, and dissolve in exactly 25 mL of diluted ethanol (4 in 5). Pipet 4 mL of this solution, add diluted ethanol (4 in 5) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances at 288 nm, A1, and A2, and at 340 nm, A12 and A22, of the sample solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of nicergoline (C24H26BrN3O3) = \( \frac{M_S \times (A_{12} - A_{22})}{(A_{12} - A_{32})} \times \frac{1}{2} \)

M3: Amount (mg) of nicergoline for assay

Dissolution Being specified separately.

Assay Weigh accurately the mass of not less than 20 Nicergoline Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of nicergoline (C24H25BrN3O3), add exactly 20 mL of a mixture of acetonitrile and water (17:3), vigorously shake for 10 minutes, centrifuge for 10 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of nicergoline for assay, previously dried in vacuum at 60°C for 2 hours, and dissolve in exactly 20 mL of the mixture of acetonitrile and water (17:3), and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A1 and A5, of nicergoline.

Amount (mg) of nicergoline (C24H26BrN3O3) = \( M_S \times \frac{A_1}{A_5} \)

M5: Amount (mg) of nicergoline for assay

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 288 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.0 with triethylamine. To 350 mL of this solution add 350 mL of methanol and 300 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of nicergoline is about 25 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nicergoline are not less than 8000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 1.0%.

Containers and storage—Containers—Tight containers.

Niceritrol

ニセリトロール

C₃₀H₃₂N₄O₈s: 556.52
Pentaerythritol tetrancionicate

[5868-05-3]

Niceritrol, when dried, contains not less than 99.0% of C₃₀H₃₂N₄O₈.

Description—Niceritrol occurs as a white to pale yellowish white powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in chloroform, solubie in N,N-dimethylformamide, very slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

Identification (1)—Determine the absorption spectrum of a solution of Niceritrol in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2)—Determine the infrared absorption spectrum of Niceritrol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point—162–165°C.

Purity (1)—Chloride—To 2.0 g of Niceritrol add 50 mL of water, and warm at 70°C for 20 minutes, while shaking occasionally. After cooling, filter, and to 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2)—Heavy metals—Proceed with 1.0 g of Niceritrol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3)—Arsenic—Prepare the test solution with 1.0 g of Niceritrol according to Method 3, and perform the test. Use 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) (not more than 2 ppm).

(4)—Pyridine—Dissolve 0.5 g of Niceritrol in N,N-dimethylformamide to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of pyridine, and add N,N-dimethylformamide to make exactly 100 mL. Pipet 1 mL of this solution, add N,N-dimethylformamide to make exactly 100 mL, then pipet 0.5 mL of this solution, add N,N-dimethylformamide to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Gas Chromatography according to the following conditions. Determine each peak area of pyridine in both solutions: the peak area of pyridine from the sample solution is not larger than the peak area of pyridine from the standard solution.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A column 3 mm in inside diameter and 3 m in length, packed with polyethylene glycol 20M for gas chromatography coated at the ratio of 10% on acid-treated and silanized siliceous earth for gas chromatography (150 to 180 μm in particle diameter).

Column temperature: A constant temperature of about 160°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of pyridine is about 2 minutes.

System suitability—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the number of theoretical steps of the peak of pyridine is not less than 1500 steps.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of pyridine is not more than 3.0%.

(5)—Free acids—Transfer about 1 g of Niceritrol, weighed accurately, to a separatory funnel, dissolve in 20 mL of chloroform, and extract with 20 mL and then 10 mL of water while shaking well. Combine the whole extracts, and titrate with 0.01 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, make any necessary correction, and calculate the amount of free acid by the following equation: it is not more than 0.1%.

Each mL of 0.01 mol/L sodium hydroxide VS equals 1.231 mg of C₉H₅NO₂.

(6)—Related substances—Dissolve 0.10 g of Niceritrol in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet exactly 2 mL of this solution, add chloroform to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (4:1) to a distance of about
10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 1 g of Nicomol, previously dried, add exactly 25 mL of 0.5 mol/L sodium hydroxide VS, boil gently for 20 minutes under a reflux condenser with a carbon dioxide absorber (soda lime). After cooling, titrate VS, boil gently for 20 minutes under a reflux condenser with 0.5 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.5 mol/L sodium hydroxide VS = 69.57 mg of C₃₄H₃₂N₄O₉

**Containers and storage** Containers—Well-closed containers.

**Nicomol**

ニコモール

[Chemical Structure Image]

C₃₄H₃₂N₄O₉: 640.64
(2-Hydroxycyclohexane-1,1,3,3-tetrayl)tetramethyl tetrancinolate

[27959-26-8]

Nicomol, when dried, contains not less than 98.0% of C₃₄H₃₂N₄O₉.

**Description** Nicomol occurs as a white, crystalline powder. It is odorless and tasteless.

It is soluble in chloroform, and practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid and in dilute nitric acid.

**Identification** (1) Mix 0.01 g of Nicomol with 0.02 g of 1-chloro-2,4-dinitrobenzene, add 2 mL of dilute ethanol, heat in a water bath for 5 minutes, cool, and add 4 mL of potassium hydroxide-ethanol TS: a dark red color develops.

(2) Dissolve 0.1 g of Nicomol in 5 mL of dilute hydrochloric acid, and add 5 drops of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the absorption spectrum of a solution of Nicomol in 1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Nicomol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <2.60> 181 – 185°C.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Nicomol in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Acidity—To 1.0 g of Nicomol add 50 mL of freshly boiled and cooled water, shake for 5 minutes, filter, and to 25 mL of the filtrate add 0.60 mL of 0.01 mol/L sodium hydroxide VS and 2 drops of phenolphthalein TS: a red color develops.

(3) Chloride <1.03>—Dissolve 0.6 g of Nicomol in 15 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.01 mol/L hydrochloric acid VS add 15 mL of dilute nitric acid and water to make 50 mL (not more than 0.024%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Nicomol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 2 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Nicomol according to Method 3, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 0.20 g of Nicomol in 20 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet 2 mL of this solution, add chloroform to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 µL of each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, ethanol (95), acetonitrile and ethyl acetate (5:3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 1.0% (1 g, 105°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 1.5 g of Nicomol, previously dried, add exactly 40 mL of 0.5 mol/L sodium hydroxide VS, boil gently under a reflux condenser connected to a carbon dioxide absorption tube (soda lime) for 10 minutes. After cooling, titrate <2.50> immediately the excess sodium hydroxide with 0.25 mol/L sulfuric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.5 mol/L sodium hydroxide VS = 80.08 mg of C₃₄H₃₂N₄O₉

**Containers and storage** Containers—Tight containers.
Nicomol Tablets

ニコモール錠

Nicomol Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of nicomol \((C_{34}H_{32}N_{4}O_{9})\) 640.64).

**Method of preparation** Prepare as directed under Tablets, with Nicomol.

**Identification** To a portion of powdered Nicomol Tablets, equivalent to 0.5 g of Nicomol according to the labeled amount, add 20 mL of chloroform, shake, and filter. Evaporate the filtrate on a water bath to dryness. Proceed with the residue as directed in the Identification (1) and (2) under Nicomol.

**Uniformity of dosage units (6.02)** It meets the requirement of the Mass variation test.

**Dissolution (6.10)** When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Nicomol Tablets is not less than 75%.

Start the test with 1 tablet of Nicomol Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add the dissolution medium to make exactly 100 mL so that each mL contains about 18 μg of nicomol \((C_{34}H_{32}N_{4}O_{9})\) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of nicomol for assay, previously dried at 105°C for 4 hours, dissolve in the dissolution medium to make exactly 100 mL, then pipet 2 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \(A_1\) and \(A_3\), of the sample solution and standard solution at 262 nm as directed under Ultraviolet-visible Spectrophotometry (2.2.24).

\[
\text{Amount (mg) of nicomol (C}_{34}\text{H}_{32}\text{N}_{4}\text{O}_{9}) = M_s \times \left(\frac{A_1}{A_3}\right) \times \frac{V}{V_T} \times \frac{1}{C} \times 18
\]

\(M_s\): Amount (mg) of nicomol for assay
\(C\): Labeled amount (mg) of nicomol \((C_{34}H_{32}N_{4}O_{9})\) in 1 tablet

**Assay** Weigh accurately not less than 20 Nicomol Tablets and powder. Weigh accurately a portion of the powder, equivalent to about 1 g of nicomol \((C_{34}H_{32}N_{4}O_{9})\), add 100 mL of 1 mol/L hydrochloric acid TS, shake well, add water to make exactly 500 mL, and filter. Discard the first 50 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 50 mL of 1 mol/L hydrochloric acid TS and water to make exactly 250 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of nicomol for assay, previously dried at 105°C for 4 hours, dissolve in 50 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add 20 mL of 1 mol/L hydrochloric acid TS and water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \(A_1\) and \(A_3\), of the sample solution and standard solution at 262 nm as directed under Ultraviolet-visible Spectrophotometry (2.2.24).

\[
M_s: \text{Amount (mg) of nicomol for assay}
\]

Nicorandil contains not less than 98.5% and not more than 101.0% of \(C_8H_9N_3O_4\), calculated on the anhydrous basis.

**Description** Nicorandil occurs as white crystals.

It is freely soluble in methanol, in ethanol (99.5) and in acetic acid (100), soluble in acetic anhydride, and sparingly soluble in water.

Melting point: about 92°C (with decomposition).

**Identification** (1) Determine the absorption spectrum of a solution of Nicorandil (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry (2.2.24), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nicorandil as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity (1)** Sulfate (1.4.15) — Dissolve 2.0 g of Nicorandil in 20 mL of dilute ethanol, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS, 20 mL of dilute ethanol and 1 mL of dilute hydrochloric acid, and dilute with water to make 50 mL (not more than 0.010%).

(2) Heavy metals (1.07) — Proceed with 2.0 g of Nicorandil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances — Dissolve 20 mg of Nicorandil in 10 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography (2.2.10) according to the following conditions, and determine each peak area by the automatic integration method: the peak area of \(N\)-(2-hydroxyethyl)isonicotinamide nitric ester, having the relative retention time of about 0.86 with respect to nicorandil, is not more than 0.5% of the peak area of nicorandil, the area of all other peaks is less than 0.1%, and
the sum area of the peaks other than nicorandil and \(N\)-(2-hydroxyethyl)isonicotinamide nitric ester is not more than 0.25% of the total peak area.

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of water, tetrahydrofuran, triethylamine and trifluoroacetic acid (982:10:5:3).
Flow rate: Adjust the flow rate so that the retention time of nicorandil is about 18 minutes.
Time span of measurement: About 3 times as long as the retention time of nicorandil beginning after the solvent peak.

**System suitability**—
Test for required detectability: Measure exactly 1 mL of the sample solution, add the mobile phase to make exactly 500 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of nicorandil obtained with 10 \(\mu\)L of this solution is equivalent to 2 to 8% of that with 10 \(\mu\)L of the solution for system suitability test.

System performance: Dissolve 10 mg of \(N\)-(2-hydroxyethyl)isonicotinamide nitric ester in the mobile phase to make 100 mL. To 1 mL of this solution add 10 mL of the mobile phase to prepare the sample solution. When the procedure is run with this solution under the above operating conditions, \(N\)-(2-hydroxyethyl)isonicotinamide nitric ester and nicorandil are eluted in this order with the resolution between these peaks being not less than 3.0.

System repeatability: When the test is repeated 6 times with 10 \(\mu\)L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of nicorandil is not more than 1.5%.

**Water** <2.48> Not more than 0.1% (2 g, volumetric titration, direct titration).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Nicorandil, dissolve in 30 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.56> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 21.12 mg of \(C_6H_5N_2O\) from the test.

**Containers and storage** Containers—Tight containers. Storage—At a temperature between 2°C and 8°C.

---

**Nicotinamide**

ニコチン酸アミド

\[
\begin{align*}
\text{C}_6\text{H}_4\text{N}_2\text{O} & : 122.12 \\
\text{Pyridine-3-carboxamide} & [98-92-0]
\end{align*}
\]

Nicotinamide, when dried, contains not less than 98.5% and not more than 102.0% of \(\text{C}_6\text{H}_4\text{N}_2\text{O}\).

**Description** Nicotinamide occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water and in ethanol (95), and slightly soluble in diethyl ether.

**Identification** (1) Mix 5 mg of Nicotinamide with 0.01 g of 1-chloro-2,4-dinitrobenzene, heat gently for 5 to 6 seconds, and fuse the mixture. Cool, and add 4 mL of potassium hydroxide-ethanol TS: a red color is produced.

(2) To 0.02 g of Nicotinamide add 5 mL of sodium hydroxide TS, and boil carefully: the gas evolved turns moistened red litmus paper blue.

(3) Dissolve 0.02 g of Nicotinamide in water to make 1000 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.34>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nicotinamide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**pH** <2.54> Dissolve 1.0 g of Nicotinamide in 20 mL of water: the pH of this solution is between 6.0 and 7.5.

**Melting point** <2.60> 128 – 131°C

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Nicotinamide in 20 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Take 0.5 g of Nicotinamide, and perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Take 1.0 g of Nicotinamide, and perform the test. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Nicotinamide according to Method 1, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(5) Readily carbonizable substances <1.15>—Take 0.20 g of Nicotinamide, and perform the test. The solution has no more color than Matching Fluid A.

**Loss on drying** <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 25 mg each of Nicotinamide
and Nicotinamide RS, both previously dried, dissolve separately in 3 mL of water, and add the mobile phase to make exactly 100 mL. Pipet 8 mL each of these solutions, and add the mobile phase to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions, and calculate the ratios, Q1 and Q2, of the peak area of nicotinamide to that of the internal standard.

\[
M = \frac{M_5 \times Q_1}{Q_2}
\]

Amount (g) of nicotinamide (C₆H₅NO₂) = \[ M \]  
Amount (mg) of dried Nicotinamide RS

**Internal standard solution**—A solution of nicotinic acid (1 in 25,000).

**Operating conditions**—
- Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 25°C.
- Mobile phase: Dissolve 1 g of sodium 1-heptane sulfonate in water to make 1000 mL. To 700 mL of this solution add 300 mL of methanol.
- Flow rate: Adjust the flow rate so that the retention time of nicotinamide is about 7 minutes.

**System suitability**—
- System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, nicotinic acid and nicotinamide are eluted in this order with the resolution between these peaks being not less than 5.
- System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nicotinamide to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

### Nicotinic Acid

ニコチン酸

C₆H₅NO₂: 123.11  
Pyridine-3-carboxylic acid [59-67-6]

Nicotinic Acid, when dried, contains not less than 99.5% of C₆H₅NO₂.

**Description** Nicotinic Acid occurs as white crystals or crystalline powder. It is odorless, and has a slightly acid taste. It is sparingly soluble in water, slightly soluble in ethanol (95), and very slightly soluble in diethyl ether.

It dissolves in sodium hydroxide TS and in sodium carbonate TS.

**Identification** (1) Triturate 5 mg of Nicotinic Acid with 0.01 g of 1-chloro-2,4-dinitrobenzene, and fuse the mixture by gentle heating for 5 to 6 seconds. Cool, and add 4 mL of potassium hydroxide-ethanol TS: a dark red color is produced.

(2) Dissolve 0.02 g of Nicotinic Acid in water to make 1000 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nicotinic Acid RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**pH** 2.54—Dissolve 0.20 g of Nicotinic Acid in 20 mL of water: the pH of this solution is between 3.0 and 4.0.

**Melting point** 2.60—234–238°C

**Purity** (1) Clarity and color of solution—Dissolve 0.20 g of Nicotinic Acid in 20 mL of water: the solution is clear and colorless.

(2) Chloride 1.03—Perform the test with 0.5 g of Nicotinic Acid. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate 1.14—Dissolve 1.0 g of Nicotinic Acid in 3 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS and 3 mL of dilute hydrochloric acid, and dilute with water to make 50 mL (not more than 0.019%).

(4) Nitro compounds—Dissolve 1.0 g of Nicotinic Acid in 8 mL of sodium hydroxide TS, and add water to make 20 mL: the solution has no more color than Matching Fluid A.

(5) Heavy metals 1.07—Proceed with 1.0 g of Nicotinic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** 2.41—Not more than 0.5% (1 g, 105°C, 1 hour).

**Residue on ignition** 2.44—Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Nicotinic Acid, previously dried, dissolve in 50 mL of water, and titrate 2.50 with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS = 12.31 mg of C₆H₅NO₂

**Containers and storage** Containers—Well-closed containers.
Nicotinic Acid Injection

Nicotinic Acid Injection is an aqueous solution for injection. It contains not less than 95.0% and not more than 110.0% of the labeled amount of nicotinic acid (C₆H₅NO₂: 123.11).

Method of preparation Prepare as directed under Injections, with Nicotinic Acid. It may contain Sodium Carbonate or Sodium Hydroxide as a solubilizer.

Description Nicotinic Acid Injection is a clear, colorless liquid. pH: 5.0 – 7.0

Identification (1) To a volume of Nicotinic Acid Injection, equivalent to 0.1 g of Nicotinic Acid according to the labeled amount, add 0.3 mL of dilute hydrochloric acid, and evaporate on a water bath to 2 mL. After cooling, collect the crystals formed, wash with small portions of ice-cold water until the last washing shows no turbidity on the addition of silver nitrate TS, and dry at 105°C for 1 hour: the crystals melt between 234°C and 238°C. With the crystals, proceed as directed in the Identification (1) under Nicotinic Acid.

(2) Dissolve 0.02 g of the dried crystals obtained in (1) in water to make 1000 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 261 nm and 263 nm, and a minimum between 235 nm and 239 nm. Separately, determine the absorbances of this solution, A₁ and A₂, at each wavelength of maximum and minimum absorption, respectively: the ratio A₂/A₁ is between 0.35 and 0.39.

Bacterial endotoxins Less than 3.0 EU/mg.

Extractable volume It meets the requirement.

Foreign insoluble matter Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter It meets the requirement.

Sterility Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Nicotinic Acid Injection, equivalent to about 0.1 g of nicotinic acid (C₆H₅NO₂), and add the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Nicotinic Acid RS, previously dried at 105°C for 1 hour, and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography: according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of nicotinic acid to that of the internal standard.

\[
S = M_S \times Q_1/Q_2
\]

Nicotinic Acid Injection is a clear, colorless liquid. pH: 5.0 – 7.0

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, nicotinic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of nicotinic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Nifedipine

C₁₇H₁₈N₂O₆: 346.33

Dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-

Cₐ₇H₇NO₆: 1162

Dissolve 0.02 g of the dried crystals obtained in (1) in water to make 1000 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 261 nm and 263 nm, and a minimum between 235 nm and 239 nm. Separately, determine the absorbances of this solution, A₁ and A₂, at each wavelength of maximum and minimum absorption, respectively: the ratio A₂/A₁ is between 0.35 and 0.39.

Bacterial endotoxins Less than 3.0 EU/mg.

Extractable volume It meets the requirement.

Foreign insoluble matter Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter It meets the requirement.

Sterility Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Nicotinic Acid Injection, equivalent to about 0.1 g of nicotinic acid (C₆H₅NO₂), and add the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Nicotinic Acid RS, previously dried at 105°C for 1 hour, and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography: according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of nicotinic acid to that of the internal standard.

\[
S = M_S \times Q_1/Q_2
\]

Nicotinic Acid Injection is a clear, colorless liquid. pH: 5.0 – 7.0

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, nicotinic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of nicotinic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Nifedipine

ニフェジピン

C₁₇H₁₈N₂O₆: 346.33

Dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-

dihydropyridine-3,5-dicarboxylate
[21829-25-4]

Nifedipine contains not less than 98.0% and not more than 102.0% of C₁₇H₁₈N₂O₆, calculated on the dried basis.

Description Nifedipine occurs as a yellow, crystalline powder. It is odorless and tasteless.

It is freely soluble in acetone and in dichloromethane, sparingly soluble in methanol, in ethanol (95) and in acetic acid (100), slightly soluble in diethyl ether, and practically insoluble in water.

It is affected by light.

Identification (1) Dissolve 0.05 g of Nifedipine in 5 mL of ethanol (95), and add 5 mL of hydrochloric acid and 2 g
of zinc powder. Allow to stand for 5 minutes, and filter. Perform the test with the filtrate as directed under Qualitative Tests <1.09> for primary aromatic amines: a red-purple color develops.

(2) Determine the absorption spectrum of a solution of Nifedipine in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Nifedipine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 172 – 175°C.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Nifedipine in 5 mL of acetone: the solution is clear and yellow.

(2) Chloride <1.07>—To 2.5 g of Nifedipine add 12 mL of dilute acetic acid and 13 mL of water, and heat to boil. After cooling, filter, and discard the first 10 mL of the filtrate. To 5 mL of the subsequent filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—To 4 mL of the filtrate obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.45 mL of 0.005 mol/L sulfuric acid VS (not more than 0.054%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of Nifedipine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Nifedipine according to Method 3, and perform the test (not more than 2 ppm).

(6) Basic substances—The procedure should be performed under protection from direct sunlight in light-resistant vessels. Dissolve 5.0 g of Nifedipine in 80 mL of a mixture of acetone and acetic acid (100) (5:3), and titrate <2.50> with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction. Not more than 1.9 mL of 0.02 mol/L perchloric acid VS is consumed.

(7) Dimethyl-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate—The procedure should be performed under protection from direct sunlight in light-resistant vessels. Dissolve 0.15 g of Nifedipine in dichloromethane to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of dimethyl-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine-dicarboxylate for thin-layer chromatography in exactly 10 mL of dichloromethane. Measure exactly 1 mL of this solution, add dichloromethane to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.00>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and ethyl acetate (3:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution, corresponding to that from the standard solution, is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.5 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay The procedure should be performed under protection from direct sunlight in light-resistant vessels. Weigh accurately about 0.12 g of Nifedipine, and dissolve in methanol to make exactly 200 mL. Measure exactly 5 mL of this solution, and add methanol to make exactly 100 mL. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 350 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of C_{19}H_{19}N_{3}O_{6} = A / 142.3 \times 40,000

Containers and storage Containers—Tight containers. Storage—Light-resistant.

**Nilvadipine**

ニルバジピン

![Chemical Structure of Nilvadipine](image)

C_{19}H_{19}N_{3}O_{6}: 385.37
3-Methyl 5-(1-methyllethyl) (4RS)-2-cyano-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate [75530-68-6]

Nilvadipine contains not less than 98.0% and not more than 102.0% of C_{19}H_{19}N_{3}O_{6}.

Description Nilvadipine occurs as a yellow crystalline powder.

It is freely soluble in acetonitrile, soluble in methanol, sparingly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Nilvadipine in acetonitrile (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Nilvadipine in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nilvadipine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nilvadipine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Nilvadipine RS: both spectra exhibit similar intensities of absorption at the same wave numbers.
Nilvadipine Tablets 1164

Melting point <2.60> 167 – 171°C

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Nilvadipine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 20 mg of Nilvadipine in 20 mL of acetonitrile, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of each related substance is not more than 0.3%, and the total of them is not more than 0.5%.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 240 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Flow rate: Adjust the flow rate so that the retention time of nilvadipine is about 12 minutes.
Time span of measurement: About 2.5 times as long as the retention time of nilvadipine beginning after the solvent peak.

System suitability—
Test for required detectability: Pipet 1 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add acetonitrile to make exactly 10 mL. Confirm that the peak area of nilvadipine obtained from 5 μL of this solution is equivalent to 7 to 13% of that from 5 μL of the solution for system suitability test.

System performance: When the procedure is run with 5 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nilvadipine is not less than 3300 and not more than 1.3, respectively.
System repeatability: Pipet 1 mL of the solution for system suitability test, and add acetonitrile to make exactly 10 mL. When the test is repeated 6 times with 5 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of nilvadipine is not more than 1.5%.

Loss on drying <2.47> Not more than 0.1% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 25 mg each of Nilvadipine and Nilvadipine RS, dissolve in methanol to make exactly 25 mL. Pipet 10 mL each of these solutions, add exactly 20 mL of the internal standard solution, 20 mL of water and methanol to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL each of the sample solution and standard solution as directed under the Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q1 and Q2, of the peak area of nilvadipine to that of the internal standard.

Amount (mg) of C19H19N3O6 = M5 × Q1/Q2

M5: Amount (mg) of Nilvadipine RS

Internal standard solution—A solution of acenaphthene in methanol (1 in 200).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 2.5 g of diammonium hydrogen phosphate in 1000 mL of water, add 10 mL of tetrabutylammonium hydroxide TS, adjust the pH to 7.0 with diluted phosphoric acid (1 in 10), and add 900 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of nilvadipine is about 12 minutes.

System suitability—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, nilvadipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nilvadipine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Nilvadipine Tablets

Nilvadipine Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of nilvadipine (C19H19N3O6: 385.37).

Method of preparation Prepare as directed under Tablets, with Nilvadipine.

Identification To a quantity of powdered Nilvadipine Tablets, equivalent to 1 mg of nilvadipine according to the labeled amount, add 100 mL of ethanol (99.5), shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry <2.24>:
It exhibits a maximum between 239 nm and 243 nm and a maximum having a broad-ranging absorption between 371 nm and 381 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Nilvadipine Tablets add V mL of a mixture of acetonitrile and water (7:3) so that each mL of the solution contains about 0.2 mg of nilvadipine (C19H19N3O6), add
exacty \( V \) mL of the internal standard solution, and disperse the particles with the aid of ultrasonic waves. Centrifuge for 10 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Nilvadipine RS, dissolve in the mixture of acetonitrile and water (7:3) to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 25 mL of the internal standard solution and the mixture of acetonitrile and water (7:3) to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay.

\[
\text{Amount (mg) of nilvadipine (C}_{19}\text{H}_{19}\text{N}_{3}\text{O}_{6}) = M_s \times \frac{Q_s}{Q_t} \times \frac{V}{100}
\]

\( M_s \): Amount (mg) of Nilvadipine RS

**Internal standard solution**—A solution of acenaphthene in acetonitrile (1 in 500).

**Dissolution**<6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Nilvadipine Tablets is not less than 85%.

Start the test with 1 tablet of Nilvadipine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 \( \mu m \). Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add exactly 1 mL of methanol, and use this solution as the sample solution. Separately, weigh accurately an amount of Nilvadipine RS, equivalent to 10 times the labeled amount of sample solution, and shake for 15 minutes, and add the mixture of acetonitrile and water (7:3) to make 50 mL. Centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Nilvadipine RS, dissolve in the mixture of acetonitrile and water (7:3) to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 25 mL of the internal standard solution and the mixture of acetonitrile and water (7:3) to make 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 mL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of nilvadipine.

\[
\text{Dissolution rate (\%)} \text{ with respect to the labeled amount of nilvadipine (C}_{19}\text{H}_{19}\text{N}_{3}\text{O}_{6}) = M_s \times \frac{A_T}{A_S} \times \frac{1}{C} \times 9
\]

\( M_s \): Amount (mg) of Nilvadipine RS

\( C \): Labeled amount (mg) of nilvadipine (C\text{19H}_{19}\text{N}_{3}\text{O}_{6}) in 1 tablet

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu m \) in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of phosphate buffer solution, pH 7.4, methanol and acetonitrile (7:7:6).

Flow rate: Adjust the flow rate so that the retention time of nilvadipine is about 5 minutes.

**System suitability**—

System performance: When the procedure is run with 5 \( \mu L \) of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nilvadipine are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nilvadipine is not more than 1.5%.

**Assay** Weigh accurately not less than 20 Nilvadipine Tablets, and powder. Weigh accurately an amount of the powder, equivalent to about 5 mg of nilvadipine (C\text{19H}_{19}\text{N}_{3}\text{O}_{6}), add 10 mL of a mixture of acetonitrile and water (7:3) and exactly 25 mL of the internal standard solution, shake for 15 minutes, and add the mixture of acetonitrile and water (7:3) to make 50 mL. Centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Nilvadipine RS, dissolve in the mixture of acetonitrile and water (7:3) to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 25 mL of the internal standard solution and the mixture of acetonitrile and water (7:3) to make 50 mL, and use this solution as the standard solution. Perform the test with 5 \( \mu L \) each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_T \) and \( Q_S \), of the peak area of nilvadipine to that of the internal standard.

\[
\text{Amount (mg) of nilvadipine (C}_{19}\text{H}_{19}\text{N}_{3}\text{O}_{6}) = M_s \times \frac{Q_T}{Q_S} \times \frac{1}{4}
\]

\( M_s \): Amount (mg) of Nilvadipine RS

**Internal standard solution**—A solution of acenaphthene in acetonitrile (1 in 500).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu m \) in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.5 g of diammonium hydrogen phosphate in 1000 mL of water, add 10 mL of tetrabutylammonium hydroxide TS, adjust the pH to 7.0 with diluted phosphoric acid (1 in 10), and add 900 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nilvadipine is about 12 minutes.

**System suitability**—

System performance: When the procedure is run with 5 \( \mu L \) of the standard solution under the above operating conditions, nilvadipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 5 \( \mu L \) of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nilvadipine to that of the internal standard is not more than 1.0%.

**Containers and storage** Contents—Well-closed containers.
Nitrazepam

Nitrazepam occurs as white to yellow crystals or crystalline powder. It is odorless.

It is freely soluble in acetic acid (100), soluble in acetone and in chloroform, slightly soluble in methanol, in ethanol (95) and in ethanol (99.5), very slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: about 227°C (with decomposition).

Identification (1) To 3 mL of a solution of Nitrazepam in methanol (1 in 500) add 0.1 mL of sodium hydroxide TS: a yellow color is produced.

(2) To 0.02 g of Nitrazepam add 15 mL of dilute hydrochloric acid, boil for 5 minutes, cool, and filter: the filtrate responds to the Qualitative Tests <1.09> for primary aromatic amines.

(3) Neutralize 0.5 mL of the filtrate obtained in (2) with sodium hydroxide TS, add 2 mL of ninhydrin TS, and heat on a water bath: a purple color is produced.

(4) Determine the absorption spectrum of a solution of Nitrazepam in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Nitrazepam in 20 mL of acetone: the solution is clear and pale yellow to light yellow in color.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Nitrazepam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.17>—Prepare the test solution with 1.0 g of Nitrazepam according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.25 g of Nitrazepam in a 10 mL of mixture of methanol and chloroform (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and chloroform (1:1) to make exactly 20 mL, pipet 2 mL of this solution, add a mixture of methanol and chloroform (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of nitromethane and ethyl acetate (17:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Nitrazepam, previously dried, and dissolve in 40 mL of acetic acid (100). Titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 28.13 mg of C15H11N3O3

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Nitrendipine

Nitrendipine occurs as a yellow crystalline powder.

It is soluble in acetonitrile, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It is gradually colored to brownish yellow by light.

A solution of Nitrendipine in acetonitrile (1 in 50) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Nitrendipine in methanol (1 in 80,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nitrendipine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 157 - 161°C.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of
Nitrendipine Tablets

**Nitrendipine Tablets**

Nitrendipine Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of nitrendipine (C\(_{18}\)H\(_{20}\)N\(_2\)O\(_6\): 360.36).

**Method of preparation** Prepare as directed under Tablets, with Nitrendipine.

**Identification** Shake a quantity of powdered Nitrendipine Tablets, equivalent to 5 mg of Nitrendipine according to the labeled amount, with 70 mL of methanol, then add methanol to make 100 mL, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (\(\lambda\) 224): it exhibits maxima between 234 nm and 238 nm, and between 350 nm and 354 nm.

**Uniformity of dosage units** (\(\leq 0.02\)) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Conduct this procedure using light-resistant vessels. To 1 tablet of Nitrendipine Tablets add 15 mL of diluted acetonitrile (4 in 5), stir until the tablet is completely disintegrated, and further stir for 10 minutes. Add diluted acetonitrile (4 in 5) to make exactly 20 mL, and centrifuge. Pipet V mL of the supernatant liquid, equivalent to about 1 mg of nitrendipine (C\(_{18}\)H\(_{20}\)N\(_2\)O\(_6\)), add exactly 5 mL of the internal standard solution, then add diluted acetonitrile (4 in 5) to make 25 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

\[ M_3 = M_5 \times Q_1/ Q_2 \times V/1 \times 1/5 \]

\(M_3\): Amount (mg) of nitrendipine for assay

**Internal standard solution**—A solution of propyl parahydroxybenzoate in diluted acetonitrile (4 in 5) (1 in 10,000).

**Dissolution** (\(\leq 0.10\)) When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of the dissolution medium containing 3 g of polysorbate 80 in 5 L of water for 5-mg tablet and the dissolution medium containing 3 g of polysorbate 80 in 2000 mL of
water for 10-mg tablet, the dissolution rate in 45 minutes of Nitrendipine Tablets is not less than 70%.

Conduct this procedure using light-resistant vessels. Start the test with 1 tablet of Nitrendipine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add the dissolution medium to make exactly V mL so that each mL contains about 5.6 μg of nitrendipine (C_{18}H_{20}N_{2}O_{6}) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of nitrendipine for assay, previously dried at 105°C for 2 hours, dissolve in methanol to make exactly 100 mL, then pipet 5 mL of this solution, and add the dissolution medium to make exactly 50 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of nitrendipine.

Dissolution rate (%) with respect to the labeled amount of nitrendipine (C_{18}H_{20}N_{2}O_{6})

= M_S × A_T/A_S × V/V × T/A × 18

M_S: Amount (mg) of nitrendipine for assay
C: Labeled amount (mg) of nitrendipine (C_{18}H_{20}N_{2}O_{6}) in 1 tablet

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 356 nm).
Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of water, tetrahydrofuran and acetonitrile (14:6:5).
Flow rate: Adjust the flow rate so that the retention time of nitrendipine is about 9 minutes.
System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nitrendipine are not less than 5000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nitrendipine is not more than 1.0%.

Containers and storage—
Containers—Tight containers.
Storage—Light-resistant.

Nitrogen

窒素

N_2: 28.01

Nitrogen is the nitrogen produced by the air liquefaction separation method.
It contains not less than 99.5 vol% of N_2.

Description—
Nitrogen is a colorless gas at room temperature and under atmospheric pressure, and is odorless.
1 mL of Nitrogen dissolves in 65 mL of water and in 9 mL of ethanol (95) at 20°C and at a pressure of 101.3 kPa.
1000 mL of Nitrogen at 0°C and at a pressure of 101.3 kPa weighs 1.251 g.

Identification—
Introduce 1 mL each of Nitrogen and nitrogen into a gas-measuring tube or syringe for gas chromatography from a cylinder with a pressure-reducing valve, through a directly connected polyvinyl chloride or stainless
Nitroglycerin Tablets

Nitroglycerin Tablets contain not less than 80.0% and not more than 120.0% of the labeled amount of nitroglycerin (C₃H₅N₃O₉). Prepare as directed under Tablets, with nitroglycerin.

Identification (1) Weigh a quantity of powdered Nitroglycerin Tablets, equivalent to 6 mg of nitroglycerin (C₃H₅N₃O₉) according to the labeled amount, shake thoroughly with 12 mL of diethyl ether, filter, and use the filtrate as the sample solution. Evaporate 5 mL of the sample solution, dissolve the residue in 1 to 2 drops of sulfuric acid, and add 1 drop of diphenylamine TS: a deep blue color develops.

(2) Evaporate 5 mL of the sample solution obtained in (1), add 5 drops of sodium hydroxide TS, heat over a low flame, and concentrate to about 0.1 mL. Cool, heat the residue with 0.02 g of potassium hydrogen sulfate: the odor of acrolein is perceptible.

Purity Free nitrate ion—Transfer an accurately measured quantity of powdered Nitroglycerin Tablets, equivalent to 20 mg of nitroglycerin (C₃H₅N₃O₉) according to the labeled amount, to a separator, add 40 mL of isopropl ether and 40 mL of water, shake for 10 minutes, and allow the layers to separate. Collect the aqueous layer, add 40 mL of isopropl ether, shake for 10 minutes, collect the aqueous layer, filter, and use the filtrate as the sample solution. Separately, transfer 10 mL of Standard Nitric Acid Solution to a separator, add 40 mL of water and 40 mL of the isopropyl ether layer of the first extraction of the sample solution, shake for 10 minutes, and use the filtrate as the sample solution. Repeat 10 mL each of the sample solution and the standard solution to Nessler tubes, respectively, heat well with 30 mL of water and 0.06 g of Griess-Romijin’s nitric acid reagent, allow to stand for 30 minutes, and observe the tubes horizontally: the sample solution has no more color than the standard solution.

Uniformity of dosage units—Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Transfer 1 tablet of Nitroglycerin Tablets to a glass-stoppered centrifuge tube, and add exactly V mL of acetic acid (100) to provide a solution containing about 30 μg of nitroglycerin (C₃H₅N₃O₉) per ml. Shake vigorously for 1 hour, and after disintegrating the tablet, centrifuge, and use the supernatant liquid as the sample solution. When the tablet does not disintegrate during this procedure, transfer 1 tablet of Nitroglycerin Tablets to a glass-stoppered centrifuge tube, wet the tablet with 0.05 mL of acetic acid (100), and grind down it with a glass rod. While rinsing the glass rod, add acetic acid (100) to make exactly V mL of a solution containing about 30 μg of nitroglycerin (C₃H₅N₃O₉) per ml. Shake for 1 hour, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 90 mg of potassium nitrate, previously dried at 105°C for 4 hours, dissolve in 5 mL of water, and add acetic acid (100) to make exactly 100 mL. Pipet 5 mL of the solution, add acetic acid (100) to make exactly 100 mL, and use this solution as the standard solution. Measure exactly 2 mL each of the sample solution and the standard solution, add 2 mL each of salicylic acid TS shake, allow to stand for 15 minutes, and add 10 mL each of water. Render the solution alkaline with about 12 mL of a solution of sodium hydroxide (2 in 5) while cooling in ice, and add water to make exactly 50 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with 2 mL of acetic acid (100) in the same manner, as the blank. Determine the absorbances, A₁ and A₂, of the subsequent solutions of the sample solution and the standard solution at 410 nm, respectively.
Nitrous Oxide

Nitrous Oxide / Official Monographs

Amount (mg) of nitroglycerin (C₃H₅N₃O₉)

\[ M_S = M_f \times \frac{A_f}{A_S} \times \frac{V}{2000} \times 0.749 \]

Disintegration §6.09

It meets the requirement, provided that the time limit of the test is 2 minutes, and the use of the disks is omitted.

Assay

Weigh accurately and disintegrate, by soft pressing, not less than 20 Nitroglycerin Tablets. Weigh accurately a portion of the powder, equivalent to about 3.5 mg of nitroglycerin (C₃H₅N₃O₉), add exactly 50 mL of acetic acid (100), shake for 1 hour, filter, and use this filtrate as the sample solution. Separately, weigh accurately about 90 mg of potassium nitrate, previously dried at 105°C, add exactly 50 mL of acetic acid (100) to make exactly 100 mL. Pipet 10 mL of the solution, add acetic acid (100) to make exactly 100 mL, and use this solution as the standard solution. Measure exactly 2 mL each of the sample solution and the standard solution, to each solution add 2 mL of salicylic acid TS, shake, allow to stand for 15 minutes, and add 10 mL of water. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry §2.245, using a solution, prepared with 2 mL of acetic acid (100) in the same manner, as the blank. Determine the absorbances, \(A_f\) and \(A_S\), of the subsequent solutions of the sample solution and the standard solution at 410 nm, respectively.

Amount (mg) of nitroglycerin (C₃H₅N₃O₉)

\[ M_S = M_f \times \frac{A_f}{A_S} \times \frac{1}{20} \times 0.749 \]

Containers and storage

Containers—Tight containers.

Storage—Light-resistant, and not exceeding 20°C.

Identification (1) A glowing splinter of wood held in Nitrous Oxide: it bursts into flame immediately.

(2) Transfer 1 mL each of Nitrous Oxide and nitrous oxide directly from metal cylinders with a pressure-reducing valve to gas measuring tubes or syringes for gas chromatography, using a polyvinyl chloride induction tube. Perform the test with these gases as directed under Gas Chromatography §2.025 according to the conditions of the Assay: the retention time of the main peak from Nitrous Oxide coincides with that of nitrous oxide.

Purity

Maintain the containers of Nitrous Oxide between 18°C and 22°C for more than 6 hours before the test, and correct the volume at 20°C and at a pressure of 101.3 kPa.

(1) Acidity or alkalinity—To 400 mL of freshly boiled and cooled water add 0.3 mL of methyl red TS and 0.3 mL of bromothymol blue TS, and boil for 5 minutes. Transfer 50 mL of this solution to each of three Nessler tubes marked A, B and C. Add 0.10 mL of 0.01 mol/L hydrochloric acid VS to tube A, 0.20 mL of 0.01 mol/L hydrochloric acid VS to tube B, stopper each of the tubes, and cool. Pass 100 mL of Nitrous Oxide through the solution in tube A for 15 minutes, employing delivery tube with an orifice approximately 1 mm in diameter and extending to within 2 mm of the bottom of the Nessler tube: the color of the solution in tube A is not deeper orange-red than that of the solution in tube B and not deeper yellow-green than that of the solution in tube C.

(2) Carbon dioxide—Pass 1000 mL of Nitrous Oxide through 50 mL of barium hydroxide TS in a Nessler tube, in the same manner as directed in (1): any turbidity produced does not exceed that produced in the following control solution.

Control solution: To 50 mL of barium hydroxide TS in a Nessler tube add 1 mL of a solution of 0.1 g of sodium hydrogen carbonate in 100 mL of freshly boiled and cooled water.

(3) Oxidizing substances—Transfer 15 mL of potassium iodide-starch TS to each of two Nessler tubes marked A and B, add 1 drop of acetic acid (100) to each of the tubes, shake, and use these as solution A and solution B, respectively. Pass 2000 mL of Nitrous Oxide through solution A for 30 minutes in the same manner as directed in (1): the color of solution A is the same as that of the stoppered, untreated solution B.

(4) Potassium permanganate-reducing substance—Pour 50 mL of water into each of two Nessler tubes marked A and B, add 0.10 mL of 0.02 mol/L potassium permanganate VS to each of the tubes, and use these as solution A and solution B, respectively. Pass 1000 mL of Nitrous Oxide through solution A in the manner as directed in (1): the color of solution A is the same as that of solution B.

(5) Chloride §1.055—Pour 50 mL of water into each of two Nessler tubes marked A and B, add 0.5 mL of silver nitrate TS to each of the tubes, shake, and use these as solution A and solution B, respectively. Pass 1000 mL of Nitrous Oxide through solution A in the same manner as directed in (1): the turbidity of solution A is the same as that of solution B.

(6) Carbon monoxide—Introduce 5.0 mL of Nitrous Oxide into a gas-cylinder or a syringe for gas chromatography...
phy from a metal cylinder holding gas under pressure and fitted with a pressure-reducing valve, through a directly connected polyvinyl tube. Perform the test with this gas according to the Gas Chromatography 2.02 under the following conditions: no peak is observed at the same retention time as that of carbon monoxide.

**Operating conditions—**

- **Detector:** A thermal-conductivity detector.
- **Column:** A column about 3 mm in inside diameter and about 3 m in length, packed with 300 to 500 \(\mu\)m zeolite for gas chromatography (0.5 nm in pore size).
- **Column temperature:** A constant temperature of about 50°C.
- **Carrier gas:** Hydrogen or helium.
- **Flow rate:** Adjust the flow rate so that the retention time of carbon monoxide is about 20 minutes.

Selection of column: To 0.1 mL each of carbon monoxide and air in a gas mixer add carrier gas to make 100 mL, and mix well. Proceed with 5.0 mL of the mixed gas under the above operating conditions. Use a column giving well-resolved peaks of oxygen, nitrogen and carbon monoxide in this order.

Detection sensitivity: Adjust the sensitivity so that the peak height of carbon monoxide obtained from 5.0 mL of the mixed gas used in the selection of column is about 10 cm.

**Assay** Withdrawing Nitrous Oxide as directed in the Purity.

Introduce 1.0 mL of Nitrous Oxide into a gas-measuring tube or syringe for gas chromatography from a metal cylinder under pressure through a pressure-reducing valve and a directly connected polyvinyl tube. Perform the test with this solution as directed under Gas Chromatography 2.02 according to the following conditions, and determine the peak area \(A_1\) of air. Separately, introduce 3.0 mL of nitrogen into a gas mixer, add carrier gas to make exactly 100 mL, mix thoroughly, and use this as the standard mixed gas. Proceed with 1.0 mL of this mixture as directed in the case of Nitrous Oxide, and determine the peak area \(A_5\) of nitrogen in the same manner.

**Operating conditions—**

- **Detector:** A thermal-conductivity detector.
- **Column:** A column about 3 mm in inside diameter and about 3 m in length, packed with silica gel for gas chromatography (300 to 500 \(\mu\)m in particle diameter).
- **Column temperature:** A constant temperature of about 50°C.
- **Carrier gas:** Hydrogen or helium.
- **Flow rate:** Adjust the flow rate so that the retention time of nitrogen is about 2 minutes.

Selection of column: To 3.0 mL of nitrogen in a gas mixer add Nitrous Oxide to make 100 mL, and mix well. Proceed with 1.0 mL of the mixed gas under the above operating conditions. Use a column giving well-resolved peaks of nitrogen and nitrous oxide in this order.

System repeatability: Repeat the test five times with the standard mixed gas under the above operating conditions: the relative standard deviation of the peak area of nitrogen is not more than 2.0%.

**Containers and storage** Containers—Metal cylinders.

Storage—Not exceeding 40°C.

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**Nizatidine**

[Nizatidine](#)

\[\text{C}_{12}\text{H}_{21}\text{N}_{5}\text{O}_{2}\text{S}_{2}: 331.46}\]

\((1\text{E})\text{Z}-\text{N}-[2-\{[(\text{Dimethylamino})\text{methyl}]\text{thiazol-4-yl}]\text{methyl}\text{ sulfanyl}]\text{ethyl}]\cdot\text{N}^\text{+}-\text{methyl}-2\text{-nitroethene-1,1-diamine}\]

\[(1\text{E})\text{Z}-\text{N}-\{2-\{[(\text{Dimethylamino})\text{methyl}]\text{thiazol-4-yl}]\text{methyl}\text{ sulfanyl}]\text{ethyl}\} \cdot \text{N}^\text{+}-\text{methyl}-2\text{-nitroethene-1,1-diamine}\]

Nizatidine, when dried, contains not less than 98.0% and not more than 101.0% of \(\text{C}_{12}\text{H}_{21}\text{N}_{5}\text{O}_{2}\text{S}_{2}\).

**Description** Nizatidine occurs as a white to pale yellowish white crystalline powder, and has a characteristic odor. It is soluble in methanol, sparingly soluble in water, and slightly soluble in ethanol (99.5).

**Identification** (1) Determine the absorption spectrum of a solution of Nizatidine in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nizatidine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the infrared absorption spectrum of Nizatidine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum or the spectrum of dried Nizatidine RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** 2.02 130 – 135°C (after drying).

**Purity** (1) Heavy metals 1.07—Proceed with 2.0 g of Nizatidine according to Method 4, and perform the test using 3 mL of sulfuric acid. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Nizatidine in 10 mL of a mixture of the mobile phase A and mobile phase B (19:6), and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mixture of the mobile phase A and mobile phase B (19:6) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 50 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography 2.02 according to the following conditions. Determine each peak area from both solutions by the automatic integration method: the area of the peaks other than nizatidine peak obtained from the sample solution is not larger than 1/5 times the nizatidine peak area from the standard solution. Furthermore, the total of the areas of peaks other than the nizatidine peak is not larger than the peak area of nizatidine from the standard solution.

**Operating conditions—**

- **Detector:** An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 5.9 g of ammonium acetate in 760 mL of water, add 1 mL of diethylamine, and adjust to pH 7.5 with acetic acid (100). To this solution add 240 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of nizatidine is about 10 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nizatidine are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nizatidine is not more than 1.0%.

Containers and storage — Containers—Tight containers.

Nizatidine Capsules

ニザチジンカプセル

Nizatidine Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of nizatidine (C12H21N5O2S2: 331.46).

Method of preparation — Prepare as directed under Capsules, with Nizatidine.

Identification — Take out the contents of Nizatidine Capsules, and powder. To a portion of the powder, equivalent to 50 mg of Nizatidine according to the labeled amount, add 50 mL of methanol, shake well, and filter. Pipet 1 mL of the filtrate, and add methanol to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry (2.24): it exhibits maxima between 239 nm and 244 nm, and between 323 nm and 327 nm.

Uniformity of dosage units — Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take out the contents from 1 capsule of Nizatidine Capsules, add the mobile phase to make V mL so that each mL contains about 1.5 mg of nizatidine (C12H21N5O2S2). After shaking vigorously for 10 minutes, centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution and add the mobile phase to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

\[
\text{Amount (mg) of nizatidine (C12H21N5O2S2)} = M_S \times \frac{Q_1}{Q_0} \times \frac{V}{10} \\
M_S: \text{Amount (mg) of Nizatidine RS}
\]

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 5.9 g of ammonium acetate in 760 mL of water, add 1 mL of diethylamine, and adjust to pH 7.5 with acetic acid (100). To this solution add 240 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of nizatidine is about 10 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nizatidine are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nizatidine is not more than 1.0%.

Containers and storage — Containers—Tight containers.

Nizatidine Capsules

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Method of preparation — Prepare as directed under Capsules, with Nizatidine.

Identification — Take out the contents of Nizatidine Capsules, and powder. To a portion of the powder, equivalent to 50 mg of Nizatidine according to the labeled amount, add 50 mL of methanol, shake well, and filter. Pipet 1 mL of the filtrate, and add methanol to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry (2.24): it exhibits maxima between 239 nm and 244 nm, and between 323 nm and 327 nm.

Uniformity of dosage units — Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take out the contents from 1 capsule of Nizatidine Capsules, add the mobile phase to make V mL so that each mL contains about 1.5 mg of nizatidine (C12H21N5O2S2). After shaking vigorously for 10 minutes, centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution and add the mobile phase to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

\[
\text{Amount (mg) of nizatidine (C12H21N5O2S2)} = M_S \times \frac{Q_1}{Q_0} \times \frac{V}{10} \\
M_S: \text{Amount (mg) of Nizatidine RS}
\]

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 5.9 g of ammonium acetate in 760 mL of water, add 1 mL of diethylamine, and adjust to pH 7.5 with acetic acid (100). To this solution add 240 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of nizatidine is about 10 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nizatidine are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nizatidine is not more than 1.0%.

Containers and storage — Containers—Tight containers.

Nizatidine Capsules

ニザチジンカプセル

Nizatidine Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of nizatidine (C12H21N5O2S2: 331.46).

Method of preparation — Prepare as directed under Capsules, with Nizatidine.

Identification — Take out the contents of Nizatidine Capsules, and powder. To a portion of the powder, equivalent to 50 mg of Nizatidine according to the labeled amount, add 50 mL of methanol, shake well, and filter. Pipet 1 mL of the filtrate, and add methanol to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry (2.24): it exhibits maxima between 239 nm and 244 nm, and between 323 nm and 327 nm.

Uniformity of dosage units — Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take out the contents from 1 capsule of Nizatidine Capsules, add the mobile phase to make V mL so that each mL contains about 1.5 mg of nizatidine (C12H21N5O2S2). After shaking vigorously for 10 minutes, centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution and add the mobile phase to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

\[
\text{Amount (mg) of nizatidine (C12H21N5O2S2)} = M_S \times \frac{Q_1}{Q_0} \times \frac{V}{10} \\
M_S: \text{Amount (mg) of Nizatidine RS}
\]
dissolution rate in 15 minutes of Nizatidine Capsules is not
less than 80%.
Start the test with 1 capsule of Nizatidine Capsules, with-
draw not less than 10 mL of the medium at the specified
minute after starting the test, and filter through a membrane
filter with a pore size not exceeding 0.45 μm. Discard the
first 2 mL of the filtrate, pipet V mL of the subsequent fil-
trate, and add water to make exactly \( V \) mL so that each mL
contains about 10 μg of nizatidine (C₁₂H₂₁N₅O₂S₂) according
to the labeled amount. Use this solution as the sample solu-
tion. Separately, weigh accurately about 25 mg of Nizatidine
RS, previously dried at 100°C for 1 hour, and dissolve in
water to make exactly 100 mL. Pipet 2 mL of this solution,
add water to make exactly 50 mL, and use this solution as
the standard solution. Perform the test with the sample solu-
tion and standard solution as directed under Ultraviolet-
visible Spectrophotometry \( \text{<2.24>}, \) and determine the absorb-
bances, \( A_T \) and \( A_S \), at 314 nm.

Dissolution rate (%) with respect to the labeled amount
of nizatidine (C₁₂H₂₁N₅O₂S₂)
\[ M_S = \frac{A_T}{A_S} \times \frac{V}{V} \times \frac{1}{C} \times 36 \]

\( M_S: \) Amount (mg) of Nizatidine RS
\( C: \) Labeled amount (mg) of nizatidine (C₁₂H₂₁N₅O₂S₂) in 1
capsule

Assay Take out the contents of not less than 10 Nizatidine
Capsules, weigh accurately the mass of the contents, and
powder. Weigh accurately a portion of the powder, equiva-
lent to about 0.15 g of nizatidine (C₁₂H₂₁N₅O₂S₂), add ex-
4-

\[ \text{C₈H₁₁NO₃: 169.18} \]

-norepinephrine

\[ \text{C₈H₁₅NO₃: 169.18} \]

Noradrenaline, when dried, contains not less than
98.0% of \( dl \)-norepinephrine (C₈H₁₅NO₃).

Description Noradrenaline occurs as a white to light brown
or slightly reddish brown, crystalline powder.
It is freely soluble in acetic acid (100), very slightly soluble
in water, and practically insoluble in ethanol (95).
It dissolves in dilute hydrochloric acid.
It gradually changes to brown by air and by light.

Identification (1) Determine the absorption spectrum of a
solution of Noradrenaline in 0.1 mol/L hydrochloric acid TS
(3 in 100,000) as directed under Ultraviolet-visible Spectro-
photometry \( \text{<2.24>}, \) and compare the spectrum with the Ref-
ence Spectrum: both spectra exhibit similar intensities of
absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of
Noradrenaline, previously dried, as directed in the potassium
bromide disk method under Infrared Spectrophotometry
\( \text{<2.25>}, \) and compare the spectrum with the Reference Spec-
trum: both spectra exhibit similar intensities of absorption at
the same wave numbers.

Purity (1) Clarity and color of solution—Dissolve 0.10 g
of Noradrenaline in 10 mL of 0.1 mol/L hydrochloric acid
TS, and add water to make 100 mL: the solution is clear and
colorless.
(2) Arterenone—Dissolve 50 mg of Noradrenaline in
0.01 mol/L hydrochloric acid TS to make exactly 100 mL.
Determine the absorbance of the solution at 310 nm as di-
rected under Ultraviolet-visible Spectrophotometry \( \text{<2.24>}: \) it
is not more than 0.1.

(3) Adrenaline—Dissolve 10.0 mg of Noradrenaline in
2.0 mL of diluted acetic acid (100) (1 in 2). Pipet 1 mL of

Noradrenaline occurs as a white to light brown
or slightly reddish brown, crystalline powder.
It is freely soluble in acetic acid (100), very slightly soluble
in water, and practically insoluble in ethanol (95).
It dissolves in dilute hydrochloric acid.
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Identification (1) Determine the absorption spectrum of a
solution of Noradrenaline in 0.1 mol/L hydrochloric acid TS
(3 in 100,000) as directed under Ultraviolet-visible Spectro-
photometry \( \text{<2.24>}, \) and compare the spectrum with the Reference Spec-
trum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of
Noradrenaline, previously dried, as directed in the potassium
bromide disk method under Infrared Spectrophotometry
\( \text{<2.25>}, \) and compare the spectrum with the Reference Spec-
trum: both spectra exhibit similar intensities of absorption at
the same wave numbers.

Purity (1) Clarity and color of solution—Dissolve 0.10 g
of Noradrenaline in 10 mL of 0.1 mol/L hydrochloric acid
TS, and add water to make 100 mL: the solution is clear and
colorless.
(2) Arterenone—Dissolve 50 mg of Noradrenaline in
0.01 mol/L hydrochloric acid TS to make exactly 100 mL.
Determine the absorbance of the solution at 310 nm as di-
rected under Ultraviolet-visible Spectrophotometry \( \text{<2.24>}: \) it
is not more than 0.1.

(3) Adrenaline—Dissolve 10.0 mg of Noradrenaline in
2.0 mL of diluted acetic acid (100) (1 in 2). Pipet 1 mL of
Noradrenaline Injection

Noradrenaline Hydrochloride Injection

Norepinephrine Hydrochloride Injection

Norepinephrine Injection

Noradrenaline Injection is an aqueous solution for injection. It contains not less than 90.0% and not more than 110.0% of the labeled amount of dl-noradrenaline (C8H11NO3: 169.18).

Method of preparation Dissolve Noradrenaline in 0.01 mol/L hydrochloric acid TS, and prepare as directed under Injections.

Description Norepinephrine Injection is a clear, colorless liquid. It gradually becomes a pale red color by light and by air. pH: 2.3 – 5.0

Identification Transfer a volume of Noradrenaline Injection, equivalent to 1 mg of Noradrenaline according to the labeled amount, add water to make exactly 20 mL, and determine the absorbance of this solution at 310 nm as directed under Ultraviolet-visible Spectrophotometry \(2.24\): the absorbance is not more than 0.10.

(2) Adrenaline—Measure a volume of Noradrenaline Injection, equivalent to 5 mg of Noradrenaline according to the labeled amount, add 1 mL of diluted acetic acid (100) (1 in 2) and water to make exactly 10 mL, and proceed as directed in the Purity (3) under Noradrenaline.

Bacterial endotoxins \(<4.0\) Less than 300 EU/mg.

Extractable volume \(<6.0\) It meets the requirement.

Foreign insoluble matter \(<6.0\) Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter \(<6.0\) It meets the requirement.

Sterility \(<4.0\) Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Pipet a volume of Noradrenaline Injection, equivalent to about 5 mg of dl-noradrenaline (C8H11NO3), add water to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Noradrenaline Bitartrate RS, previously dried in a desiccator (in vacuum, silica gel) for 24 hours, dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Pipet 5 mL of each of the sample solution and the standard solution, add 0.2 mL each of starch TS, then add iodine TS dropwise with swirling until a persistent blue color is produced. Add 2 mL of iodine TS, and shake. Adjust the pH of the solution to 6.5 with 0.05 mol/L disodium hydrogenphosphate TS, add 10 mL of phosphate buffer solution, pH 6.5, and shake. Immediately after allowing to stand for 3 minutes, add sodium thiosulfate TS dropwise until a persistent blue color is produced.

\[ \text{Amount (mg) of } dl\text{-noradrenaline (C}_8\text{H}_11\text{NO}_3) = M_5 \times \frac{A_5}{A_3} \times 0.502 \]

\(M_5\): Amount (mg) of Noradrenaline Bitartrate RS

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.
Norethisterone

ノルエチステロン

C_{20}H_{26}O_2: 298.42
17-Hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one
[68-22-4]

Norethisterone, when dried, contains not less than 97.0% and not more than 103.0% of C_{20}H_{26}O_2.

Description Norethisterone occurs as a white to pale yellowish white crystalline powder. It has no odor.

It is sparingly soluble in ethanol (95), in acetone, and in tetrahydrofuran, slightly soluble in diethyl ether, and very slightly soluble in water.

It is affected by light.

Identification (1) To 2 mg of Norethisterone add 2 mL of sulfuric acid: the solution shows a red-brown color and a yellow-green fluorescence. Add 10 mL of water to this solution cautiously: a yellow color develops and a yellow-brown precipitate is formed.

(2) To 25 mg of Norethisterone add 3.5 mL of a solution of 0.05 g of hydroxylammonium chloride and 0.05 g of anhydrous sodium acetate trihydrate in 25 mL of methanol. Heat under a reflux condenser on a water bath for 5 hours, cool, and add 15 mL of water. Collect the precipitate formed, wash with 1 to 2 mL of water, recrystallize from tetrahydrofuran, slightly soluble in diethyl ether, and very slightly soluble in water.

Optical rotation [α]D: −32° to −37° (after drying, 0.25 g, acetone, 25 mL, 100 mm).

Melting point <2.60> 203 – 209°C.

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacuum, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.2 g of Norethisterone, previously dried, dissolve in 40 mL of tetrahydrofuran, add 10 mL of a solution of silver nitrate (1 in 20), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 29.84 mg of C_{20}H_{26}O_2

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Norfloxacin

ノルフロキサシン

C_{16}H_{18}FN_{3}O_{3}: 319.33
1-Ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid [70458-96-7]

Norfloxacin, when dried, contains not less than 99.0% of C_{16}H_{18}FN_{3}O_{3}.

Description Norfloxacin occurs as a white to pale yellow crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in ethanol (99.5) and in acetone, very slightly soluble in methanol, and practically insoluble in water.

It dissolves in dilute hydrochloric acid TS and in sodium hydroxide TS.

It is hygroscopic.

It is gradually colored by light.

Identification (1) Dissolve 0.01 g of Norfloxacin in a solution of sodium hydroxide (1 in 250) to make 100 mL. To 5 mL of this solution add a solution of sodium hydroxide (1 in 250) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.44>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve a suitable amount of Norfloxacin in a suitable amount of acetone, evaporate the acetone under reduced pressure, and dry the residue. Determine the infrared absorption spectrum of the residue so obtained as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Sulfate <1.14>—Dissolve 1.0 g of Norfloxacin in 7 mL of 0.5 mol/L sodium hydroxide TS and 23 mL of water, and add 1 drop of phenolphthalein TS. Add gradually dilute hydrochloric acid (1 in 3) to this solution until the red color disappears, then add 0.5 mL of dilute hydrochloric acid, and cool in ice for 30 minutes. Filter through a glass filter (G4), and wash the residue with 10 mL of water. Combine the filtrate and the washing, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows. To 0.50 mL of 0.005 mol/L sulfuric acid VS add 7 mL of 0.5 mol/L sodium hydroxide TS and 1 drop of phenolphthalein TS, add dilute hydrochloric acid (1 in 3) until the red color disappears, then add 1.5 mL of dilute hydrochloric acid, 1 or 2 drops of bromphenol blue TS and water to make 50 mL (not more than 0.024%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Norfloxacin according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution
(not more than 15 ppm).

(3) Arsenic <1.10>—Prepare the test solution with 1.0 g of Norfloxacin according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.10 g of Norfloxacin in 50 mL of a mixture of methanol and acetone (1:1), and use this solution as the sample solution.

Pipet 1 mL of the sample solution, add a mixture of methanol and acetone (1:1) to make exactly 100 mL. Pipet 2 mL of this solution, add a mixture of methanol and acetone (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography (5 - 7 μm in particle diameter). Develop with a mixture of dichloromethane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm and 366 nm): the number of the spot other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Norfloxacin, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

\[
\text{Each mL of 0.1 mol/L perchloric acid VS} = 31.93 \text{ mg of C}_{16}\text{H}_{18}\text{FN}_{3}\text{O}_{3}
\]

Containers and storage Containers—Light-resistant. Storage—Light-resistant.

Norgestrel

ノルゲストレル

\[
\begin{align*}
\text{C}_{21}\text{H}_{28}\text{O}_{2} & : 312.45 \\
13\text{-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one} & \ [6533-00-2]
\end{align*}
\]

Norgestrel, when dried, contains not less than 98.0% of C_{21}H_{28}O_{2}.

Description Norgestrel occurs as white crystals or crystalline powder.

It is soluble in tetrahydrofuran and in chloroform, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

Identification (1) Dissolve 1 mg of Norgestrel in 2 mL of ethanol (95), and add 1 mL of sulfuric acid: a red-purple color develops. With this solution, examine under ultraviolet light (main wavelength: 365 nm): the solution shows a red-orange fluorescence.

(2) Determine the infrared absorption spectrum of Norgestrel, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 206 – 212°C.

Purity (1) Heavy metals <1.07>—Take 1.0 g of Norgestrel, heat gently to carbonize, cool, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and ignite the ethanol to burn. After cooling, add 1 mL of sulfuric acid, proceed with this solution according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 30 mg of Norgestrel in 5 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography (5 - 7 μm in particle diameter). Develop with a mixture of dichloromethane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (0.5 g).

Assay Weigh accurately about 0.2 g of Norgestrel, previously dried, dissolve in 40 mL of tetrahydrofuran, add 10 mL of a solution of silver nitrate hexahydrate in ethanol (95) (1 in 10), and ignite the ethanol to burn. After cooling, add 1 mL of sulfuric acid, proceed with this solution according to Method 4, and perform the test. Prepare the control solution with 0.6 mL of Standard Lead Solution (not more than 20 ppm).

Perform a blank determination, and make any necessary correction.

\[
\text{Each mL of 0.1 mol/L sodium hydroxide VS} = 31.25 \text{ mg of C}_{21}\text{H}_{28}\text{O}_{2}
\]

Containers and storage Containers—Well-closed containers.
Norgestrel and Ethinylestradiol Tablets

ノルゲストレル・エチニルエストラジオール錠

Norgestrel and Ethinylestradiol Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of norgestrel (C₂₁H₂₈O₂: 312.45) and ethinylestradiol (C₂₀H₂₄O₂: 296.40).

Method of preparation
Prepare as directed under Tablets, with Norgestrel and Ethinylestradiol.

Identification
Weigh a quantity of Norgestrel and Ethinylestradiol Tablets, equivalent to 10 mg of Norgestrel according to the labeled amount, previously powdered, add 10 mL of chloroform, shake for 10 minutes, and filter. To 2 mL of the filtrate add 6 mL of sodium hydroxide TS, shake vigorously, and centrifuge. Take 1 mL of the chloroform layer, evaporate on a water bath to dryness, dissolve the residue in 2 mL of ethanol (95%), and add 1 mL of sulfuric acid: a red-purple color develops. Examine under ultraviolet light (main wavelength: 365 nm): this solution shows a red-orange fluorescence (norgestrel).

(2) Take 1 mL of the filtrate obtained in (1), evaporate on a water bath to dryness, add 1 mL of boric acid-methanol buffer solution to the residue, shake, and cool in ice. Add 1 mL of ice-cold diazo TS, shake, add 1 mL of sodium hydroxide TS, and shake: a red-orange color develops (ethinylestradiol).

(3) Use the filtrate obtained in (1) as the sample solution. Separately, dissolve 10 mg of Norgestrel RS and 1 mg of Ethinylestradiol RS, respectively, in 10 mL of chloroform, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography 2.07. Spot 20 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane, methanol and water (368:32:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of p-toluene-sulfonate in ethanol (95%) (1 in 5) on the plate, and heat at 105°C for 5 minutes. Examine under ultraviolet light (main wavelength: 365 nm): two spots from the sample solution show the similar color tone and Rf value to each spot from the standard solutions (1) and (2).

Uniformity of dosage units 6.02 Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Add 2 mL of diluted methanol (7 in 10) to 1 tablet of Norgestrel and Ethinylestradiol Tablets, add exactly 2 mL of the internal standard solution, shake for 20 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with pore size of not more than 0.2 μm, and use this filtrate as the sample solution. Separately, weigh accurately quantities of Norgestrel RS and of Ethinylestradiol RS, equivalent to 100 times each of the labeled amounts, dissolve in diluted methanol (7 in 10) to make exactly 200 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.07 according to the following conditions. Calculate the ratios, Q₁ and Q₂, of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the sample solution and also the ratios, Qₐ and Qₐ₀, of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the standard solution.

\[ M_{S_a} = \frac{V_s \times C_{S_a}}{Q_{S_a} \times Q_{100}} \]

\[ M_{S_b} = \frac{V_s \times C_{S_b}}{Q_{S_b} \times Q_{100}} \]

Dissolution rate (%) with respect to the labeled amount of norgestrel (C₂₁H₂₈O₂)

\[ = M_{S_a} \times A_{S_a} \times 1/V \times 1/C_3 \times 54 \]

Dissolution rate (%) with respect to the labeled amount of ethinylestradiol (C₂₀H₂₄O₂)

\[ = M_{S_b} \times A_{S_b} \times 1/V \times 1/C_3 \times 54 \]

Mₐ: Amount (mg) of Norgestrel RS
Mₐ₀: Amount (mg) of Ethinylestradiol RS

Internal standard solution—A solution of diphenyl in diluted methanol (7 in 10) (1 in 50,000).

Operating conditions—
Proceed as directed in the operating conditions in the Assay.

System suitability—
Proceed as directed in the system suitability in the Assay.

Dissolution 6.10 When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Norgestrel and Ethinylestradiol Tablets is not less than 70%.

Start the test with 1 tablet of Norgestrel and Ethinylestradiol Tablets, withdraw not less than 30 μL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet exactly V mL of the subsequent filtrate, equivalent to about 17 μg of norgestrel (C₂₁H₂₈O₂) and about 1.7 μg of ethinylestradiol (C₂₀H₂₄O₂), transfer into a chromatography column [prepared by packing 0.36 g of octadecylsilanized silica gel for pretreatment (55 to 105 μm in particle diameter) in a tube about 1 cm in inside diameter]. After washing the column with 15 mL of water, elute with 3 mL of methanol, and evaporate the effluent in a water bath to dryness at about 40°C with the aid of a current air. Dissolve the residue in exactly 2 mL of diluted methanol (7 in 10), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Norgestrel RS and about 2.5 mg of Ethinylestradiol RS, dissolve in diluted methanol (7 in 10) to make exactly 100 mL, then pipet 3 mL of this solution, add diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.07 according to the following conditions. Determine the peak areas, A₁ and A₂, of norgestrel and ethinylestradiol from the sample solution, and the peak areas, Aₐ and Aₐ₀, of norgestrel and ethinylestradiol from the standard solution.

\[ = M_{S_a} \times A_{S_a} \times 1/V \times 1/C_3 \times 54 \]
Nortriptyline Hydrochloride / Official Monographs

Assay
Weigh accurately not less than 20 Norgestrel and Ethinylestradiol Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 1 mg of norgestrel (C₂₁H₂₈O₂), add 4 mL of diluted methanol (7 in 10), add exactly 4 mL of the internal standard solution, shake for 20 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with pore size of not more than 0.2 μm, and use this filtrate as the sample solution. Separately, weigh accurately about 50 mg of Norgestrel RS and about 5 mg of Ethinylestradiol RS, and dissolve in diluted methanol (7 in 10) to make exactly 200 mL. Pipet 4 mL of this solution, add exactly 4 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography to determine the relative standard deviation of the ratios, the relative standard deviation of the ratios of the peak area of ethinylestradiol and norgestrel to that of the internal standard are not more than 1.0%, respectively.

Containers and storage
Containers—Tight containers.

Nortriptyline Hydrochloride
ノルトリプチリン塩酸塩

C₁₉H₂₁N.HCl: 299.84
3-(10,11-Dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-N-methylpropylamine monohydrochloride [894-71-3]

Nortriptyline Hydrochloride, when dried, contains not less than 98.5% of C₁₉H₂₁N.HCl.

Description
Nortriptyline Hydrochloride occurs as a white or yellowish white, crystalline powder. It is odorless, or has a faint, characteristic odor.

It is freely soluble in acetic acid (100) and in chloroform, soluble in ethanol (95), sparingly soluble in water, and practically insoluble in diethyl ether.

The pH of a solution of Nortriptyline Hydrochloride (1 in 100) is about 5.5.

Melting point: 215 – 220°C.

Identification
(1) To 5 mL of a solution of Nortriptyline Hydrochloride (1 in 100) add 1 mL of bromine TS: the color of the test solution disappears.

(2) To 5 mL of a solution of Nortriptyline Hydrochloride (1 in 100) add 1 to 2 drops of a solution of quinhydrone in ethanol (1 in 100): a red color gradually develops.

(3) Determine the absorption spectrum of a solution of Nortriptyline Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Nortriptyline Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(5) A solution of Nortriptyline Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> for chloride.

Purity
(1) Clarity and color of solution—Dissolve 0.10 g of Nortriptyline Hydrochloride in 10 mL of water: the solution is clear and colorless to very light yellow.

(2) Heavy metals <1.67>—Proceed with 1.0 g of Nortriptyline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Nortriptyline Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

System suitability—
Proceed as in the operating conditions in the Assay.

System suitability—
Proceed as in the operating conditions in the Assay.
(4) Related substances—Dissolve 0.50 g of Nortriptyline Hydrochloride in 20 mL of chloroform, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add chloroform to make exactly 50 mL. Pipet 5 mL of this solution, add chloroform to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 4 mL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, methanol and diethylamine (8:1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Nortriptyline Hydrochloride, previously dried, dissolve in 5 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate (100), add 50 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 4 mL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, methanol and diethylamine (8:1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Each mL of 0.1 mol/L perchloric acid VS = 29.98 mg of C_{19}H_{21}N.HCl.

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.

Narcotine

ノスカピン

C_{22}H_{25}NO_5: 413.42
(35)-6,7-Dimethoxy-3-[(5R)-4-methoxy-6-methyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isouquinolin-5-yl]isobenzofuran-1(5H)-one

[128-62-1]

Noscapine, when dried, contains not less than 98.5% of C_{22}H_{25}NO_5.

Description Noscapine occurs as white crystals or crystalline powder. It is odorless and tasteless.

It is very soluble in acetic acid (100), slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Noscapine in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.249>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Noscapine, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]_D^0: +42° to +48° (after drying, 0.5 g, 0.1 mol/L hydrochloric acid TS, 25 mL, 100 nm).

Melting point <2.60> 174 – 177°C.

Purity (1) Chloride <1.03>—Dissolve 0.7 g of Noscapine in 20 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test with this solution. Prepare the control solution as follows: To 0.4 mL of 0.01 mol/L hydrochloric acid add 20 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.02%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Noscapine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Morphine—Dissolve 10 mg of Noscapine in 1 mL of water and 5 mL of 1-nitroso-2-naphthol TS with shaking, add 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. Add 1 mL of a solution of sodium nitrite (1 in 5000), and warm at 40°C for 5 minutes. After cooling, shake the solution with 10 mL of chloroform, centrifuge, and collect the aqueous layer: the solution so obtained has no more color than a pale red.

(4) Related substances—Dissolve 0.7 g of Noscapine in 50 mL of acetone, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add acetone to make exactly 50 mL. Pipet 5 mL of this solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene, ethanol (99.5) and ammonia solution (28) (60:60:9:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute bismuth subnitrate-potassium iodide TS for spray on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (2 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.8 g of Noscapine, previously dried, dissolve in 30 mL of acetic acid (100) and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 41.34 mg of C_{22}H_{25}NO_5.
Containers and storage  Containers—Well-closed containers.
Storage—Light-resistant.

Noscapine Hydrochloride Hydrate

**Narcotine Hydrochloride Hydrate**

Noscapine Hydrochloride Hydrate, when dried, contains not less than 98.0% of noscapine hydrochloride C₂₂H₂₃NO₇.HCl: 449.88.

**Description**  Noscapine Hydrochloride Hydrate occurs as colorless or white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water, in acetic acid (100), and in acetic anhydride, soluble in ethanol (95), and practically insoluble in diethyl ether.

**Identification (1)**  To 1 mg of Noscapine Hydrochloride Hydrate add 1 drop of formaldehyde-sulfuric acid TS: a purple color, changing to yellow-brown, is produced.

(2)  To 1 mg of Noscapine Hydrochloride Hydrate add 1 drop of a solution of ammonium vanadate (V) in sulfuric acid (1 in 20): a purple color is produced.

(3)  Dissolve 0.02 g of Noscapine Hydrochloride Hydrate in 1 mL of water, and add 3 drops of sodium acetate TS: a white, flocculent precipitate is produced.

(4)  Dissolve 1 mg of Noscapine Hydrochloride Hydrate in 1 mL of diluted sulfuric acid (1 in 35), shake with 5 drops of a solution of disodium chloromotropate dihydrate (1 in 50), and add 2 mL of sulfuric acid dropwise: a purple color is produced.

(5)  Dissolve 0.1 g of Noscapine Hydrochloride Hydrate in 10 mL of water, make the solution alkaline with ammonia TS, and shake with 10 mL of chloroform. Separate the chloroform layer, wash with 5 mL of water, and filter. Distil most of the filtrate on a water bath, add 1 mL of ethanol (99.5), and evaporate to dryness. Dry the residue at 105° to 110°C, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of disodium chloromotropate dihydrate C₇H₆NO₈ (1 in 50) and 1 mL of dilute perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS is equivalent to 44.99 mg of C₂₂H₂₃NO₇.HCl.

**Containers and storage**  Containers—Well-closed containers.
Storage—Light-resistant.

**Nystatin**

Nystatin is a mixture of polyene macrolide substances having antifungal activity produced by the growth of *Streptomyces noursei*.

It contains not less than 4600 units (potency) per mg, calculated on the dried basis. The potency of Nystatin is expressed as the unit of nystatin (C₄₇H₇₅NO₁₇: 926.09), and one unit corresponds to 0.27 μg of nystatin (C₄₇H₇₅NO₁₇).

**Description**  Nystatin occurs as a white to light yellow-brown powder.

It is soluble in formamide, sparingly soluble in methanol, slightly soluble in ethanol (95), and very slightly soluble in water.

It dissolves in sodium hydroxide TS.

**Identification (1)**  Dissolve 1 mg of Nystatin in 5 mL of water and 1 mL of sodium hydroxide TS, heat for 2 minutes, and cool. To this solution add 3 mL of a solution of 4-aminoacetophenone in methanol (1 in 200) and 1 mL of hydrochloric acid: a red-purple color develops.

(2)  To 10 mg of Nystatin add 50.25 mL of a mixture of diluted methanol (4 in 5) and sodium hydroxide TS (200:1), heat at not exceeding 50°C to dissolve, then add diluted methanol (4 in 5) to make 500 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nystatin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity**  Heavy metals <1.07%—Proceed with 1.0 g of Nystatin according to Method 4, and perform the test. Prepare the
control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** Not more than 5.0% (0.3 g, in vacuum, 60°C, 3 hours).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Saccharomyces cerevisiae* ATCC 9763

(ii) Culture medium—Use the medium 2) Medium for test organism [12] under (1) Agar media for seed and base layer.

(iii) Standard solutions—Use a light-resistant container. Weigh accurately an amount of Nystatin RS equivalent to about 60,000 units, previously dried at 40°C for 2 hours in vacuum (not more than 0.67 kPa), dissolve in formamide to make a solution of 3000 units per mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 300 units and 150 units, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Use a light-resistant container. Weigh accurately an amount of Nystatin equivalent to about 60,000 units, previously dried at 40°C for 2 hours in vacuum (not more than 0.67 kPa), dissolve in formamide to make a solution of 3000 units per mL, and use this solution as the standard stock solution. Take exactly a suitable amount of the sample stock solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 300 units and 150 units, and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

**Containers and storage** Containers—Tight containers. Storage—Light-resistant, and in a cold place.

**Ofloxacin**

オフロキサシン

C₁₈H₂₀FN₃O₄·3·6·1·₃₇

(3RS)-9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid

[82419-36-1]

Ofloxacin, when dried, contains not less than 99.0% and not more than 101.0% of ofloxacin (C₁₈H₂₀FN₃O₄).

**Description** Ofloxacin occurs as pale yellowish white to light yellowish white, crystals or crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in water, and very slightly soluble in acetonitrile and in ethanol (99.5).

A solution of Ofloxacin in sodium hydroxide TS (1 in 20) does not show optical rotation.

It is changed in color by light.

Melting point: about 263°C (with decomposition).

**Identification** (1) Determine the absorption spectrum of a solution of Ofloxacin in 0.1 mol/L hydrochloric acid TS (1 in 150,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ofloxacin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** (1) Heavy metals <1.0%>—Proceed with 2.0 g of Ofloxacin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure without exposure to light. Dissolve 10 mg of Ofloxacin in 50 mL of a mixture of water and acetonitrile (6:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and acetonitrile (6:1) to make exactly 20 mL. Pipet 1 mL of this solution, add a mixture of water and acetonitrile (6:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than ofloxacin obtained from the sample solution is not larger than 0.4 times the peak area of ofloxacin from the standard solution, and the total area of the peaks other than ofloxacin is not larger than the peak area from the standard solution.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 294 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: Dissolve 7.0 g of sodium perchlorate monohydrate and 4.0 g of ammonium acetate in 1300 mL of water, adjust the pH to 2.2 with phosphoric acid, and add 240 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ofloxacin is about 20 minutes.

Time span of measurement: About 1.8 times as long as the retention time of ofloxacin beginning after the solvent peak.

**System suitability**—

Test for required detectability: Measure 1 mL of the standard solution, and add a mixture of water and acetonitrile (6:1) to make exactly 20 mL. Confirm that the peak area of ofloxacin obtained from 10 μL of this solution is equivalent to 4 to 6% of that from 10 μL of the standard solution.

System performance: To 0.5 mL of the sample solution add 1 mL of a solution of ofloxacin demethyl substance in a mixture of water and acetonitrile (6:1) (1 in 20,000) and a
mixture of water and acetonitrile (6:1) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ofloxacin demethyl substance and ofloxacin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ofloxacin is not more than 2.0%.

**Loss on drying** <2.44> Not less than 0.2% (1 g, 105°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Ofloxacin, previously dried, dissolve in 100 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 36.14 mg of C18H20FN3O4

**Containers and storage** Containers—Tight containers.

**Olive Oil**

**Oleum Olivae**

オリブ油

Olive Oil is the fixed oil obtained by expression from the ripe fruit of *Olea europaea* Linné (*Oleaceae*).

**Description** Olive Oil is a light yellow oil. It has a faint odor, which is not rancid, and has a bland taste.

It is miscible with diethyl ether, with petroleum diethyl ether and with carbon disulfide.

It is slightly soluble in ethanol (95).

The whole or a part of it congeals between 0°C and 6°C. Congealing point of the fatty acids: 17 – 26°C

**Specific gravity** <1.13> α<sup>20</sup>: 0.908 – 0.914

**Acid value** <1.13> Not more than 1.0.

**Saponification value** <1.13> 186 – 194

**Unsaponifiable matters** <1.13> Not more than 1.5%.

**Iodine value** <1.13> 79 – 88

**Purity** (1) Drying oil—Mix 2 mL of Olive Oil with 10 mL of diluted nitric acid (1 in 4), add 1 g of powdered sodium nitrite little by little with thorough shaking, and allow to stand in a cold place for 4 to 10 hours: the mixture congeals to a white solid.

(2) Peanut oil—Weigh exactly 1.0 g of Olive Oil, dissolve in 60 mL of sulfuric acid-hexane-methanol TS, boil for 2.5 hours on a water bath under a reflux condenser, cool, transfer to a separator, and add 100 mL of water. Wash the flask with 50 mL of petroleum ether, add the washing to the separator, shake, allow to stand, and separate the petroleum ether layer. Extract the water layer with another 50 mL of petroleum ether, and combine the petroleum ether layer with the former petroleum ether solution. Wash the petroleum ether solution repeatedly with 20-mL portions of water until the washings show no more acidity to methyl orange TS. Then add 5 g of anhydrous sodium sulfate, shake, filter, wash anhydrous sodium sulfate with two 10-mL portions of petroleum ether, filter the washings using the former separator, combine the filtrates, distil the petroleum ether on a water bath, passing nitrogen. Dissolve the residue in acetone to make exactly 20 mL, and use this solution as the sample solution. Separately, dissolve 0.067 g of methyl behenate in acetone to make exactly 50 mL. Pipet 2 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions. Measure the peak heights, *H*<sub>T</sub> and *H*<sub>S</sub>, of methyl behenate of respective solutions: *H*<sub>T</sub> is not higher than *H*<sub>S</sub>.

**Operating conditions**—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with silanized siliceous earth for gas chromatography (150 to 180 μm in particle diameter), coated with polyethylene glycol 20 mol/L in a ratio of 5%.

Column temperature: A constant temperature of about 220°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of methyl behenate is about 18 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of methyl behenate obtained from 2 μL of the standard solution is 5 to 10 mm.

**Containers and storage** Containers—Tight containers.

**Omeprazole**

オメプラゾール

- **C<sub>17</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>S: 345.42**
- **RS)-5-Methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfinyl]-1H-benzoimidazole**

Omeprazole, when dried, contains not less than 99.0% and not more than 101.0% of C<sub>17</sub>H<sub>19</sub>N<sub>2</sub>O<sub>4</sub>S.

**Description** Omeprazole occurs as a white to yellowish white crystalline powder.

It is freely soluble in *N,N*-dimethylformamide, sparingly soluble in ethanol (99.5), and practically insoluble in water. A solution of Omeprazole in *N,N*-dimethylformamide (1 in 25) shows no optical rotation.

It gradually turns yellowish white on exposure to light.

**Melting point:** about 150°C (with decomposition).

**Identification** (1) Add phosphate buffer solution, pH
7.4, to 1 mL of a solution of Omeprazole in ethanol (99.5) (1 in 1000) to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

2. Determine the infrared absorption spectrum of Omeprazole as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Omeprazole in 25 mL of N,N-dimethylformamide: the solution is clear and colorless or light yellow. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance at 420 nm is not more than 0.3.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Omeprazole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

3. Related substances—Conduct the procedure soon after preparation of the sample solution. Dissolve 50 mg of Omeprazole in 50 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.07> according to the following conditions. Determine each of the peak areas of the sample solution by the automatic integration method, and calculate the amounts of them by the area percentage method: each of the amount of the peaks other than omeprazole is not more than 0.1%, and the total amount of the peaks other than omeprazole is not more than 0.5%.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 2.83 g of disodium hydrogen phosphate dodecahydrate and 0.21 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL. If necessary, adjust the pH to 7.6 with diluted phosphoric acid (1 in 100). Add 11 volumes of acetonitrile to 29 volumes of this solution.
Flow rate: Adjust the flow rate so that the retention time of Omeprazole is about 8 minutes.
Time span of measurement: About 10 times as long as the retention time of omeprazole, beginning after the solvent peak.

System suitability—
Test for required detectability: Pipet 5 mL of the sample solution, and add the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add the mobile phase to make exactly 25 mL. Confirm that the peak area of omeprazole obtained from 10 μL of this solution is equivalent to 15 to 25% of that from 10 μL of the solution for system suitability test.

System performance: Dissolve 10 mg of Omeprazole and 25 mg of 1,2-dinitrobenzene in 5 mL of sodium borate solution (19 in 5000) and 95 mL of ethanol (99.5). If the procedure is run with 10 μL of this solution under the above conditions, omeprazole and 1,2-dinitrobenzene are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of omeprazole is not more than 2.0%.

Loss on drying <2.41> Not more than 0.2% (1 g, in vacuum, phosphorus (V) oxide, 50°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Omeprazole, previously dried, dissolve in 70 mL of N,N-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Separately, perform a blank determination using the same method on a solution consisting of 70 mL of N,N-dimethylformamide and 12 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS = 34.54 mg of C17H19N3O3S

Containers and storage Containers—Tight containers.
Storage—Light-resistant, in a cold place.

Powdered Opium

Opium Pulveratum

アヘン末

Powdered Opium is a homogeneous powder of opium obtained from Papaver somniferum Linné (Papaveraceae). Starch or Lactose Hydrate may be added.

Powdered Opium contains not less than 9.5% and not more than 10.5% of morphine (C17H19NO3: 285.34).

Description Powdered Opium occurs as a yellow-brown to dark brown powder.

Identification (1) To 0.1 g of Powdered Opium add 5 mL of diluted ethanol (7 in 10), dissolve by treating with ultrasonic waves for 10 minutes, and add diluted ethanol (7 in 10) to make 10 mL. Filter this solution, and use the filtrate as the sample solution. Separately, dissolve 25 mg of Morphine Hydrochloride Hydrate, 12 mg of Codeine Phosphate Hydrate, 2 mg of Papaverine Hydrochloride, and 12 mg of Noscapine Hydrochloride Hydrate separately in 25 mL of diluted ethanol (7 in 10), and use these solutions as the standard solution (1), the standard solution (2), the standard solution (3) and the standard solution (4), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 μL each of the sample solution and standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene, ethanol (99.5) and ammonia water (28)
Diluted Opium Powder

アヘン散

Diluted Opium Powder contains not less than 0.90% and not more than 1.10% of morphine (C17H19NO3: 285.34).

**Method of preparation**

<table>
<thead>
<tr>
<th>Powdered Opium</th>
<th>100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch or a suitable diluent</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 g

Prepare as directed under Powders, with the above ingredients. Lactose hydrate should not be used.

**Description** Diluted Opium Powder occurs as a light brown powder.

**Identification (1)** Proceed with 1 g of Diluted Opium Powder as directed in the Identification (1) under Powdered Opium.

**Identification (2)** Proceed with 1 g of Diluted Opium Powder as directed in the Identification (2) under Powdered Opium.

**Assay** Place about 50 g of Diluted Opium Powder, accurately weighed, in a glass-stoppered flask, and stir with 250 mL of dilute ethanol in a water bath at 40°C for 1 hour. Filter the mixture through a glass filter (G3). Transfer the residue on the filter to the first glass-stoppered flask, and add 50 mL of dilute ethanol. Stir the mixture in a water bath at 40°C for 10 minutes, and filter through the same glass filter. Repeat the extraction with three 50-mL portions of dilute ethanol. Evaporate the combined filtrate in a mortar to dryness on a water bath. Add 10 mL of ethanol (99.5) to the residue, evaporate to dryness again, and, after cooling, triturate it with exactly 10 mL of water. Proceed with this solution as directed in Assay under Powdered Opium.

Each mL of 0.05 mol/L sulfuric acid VS = 28.53 mg of C17H19NO3

**Containers and storage** Containers—Tight containers.

Opium Tincture

アヘンチンキ

Opium Tincture contains not less than 0.93 w/v% and not more than 1.07 w/v% of morphine (C17H19NO3: 285.34).

**Method of preparation**

<table>
<thead>
<tr>
<th>Powdered Opium</th>
<th>100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>35 vol% Ethanol</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Prepare as directed under Tinctures, with the above ingredients. May be prepared with an appropriate quantity of Ethanol and Purified Water or Purified Water in Containers in place of 35 vol% Ethanol.

**Description** Opium Tincture is a dark red-brown liquid.
Opium Alkaloids Hydrochlorides

アヘンアルカロイド塩酸塩

Opium Alkaloids Hydrochlorides consist of the hydrochlorides of some of the main alkaloids obtained from opium.

It contains not less than 47.0% and not more than 52.0% of morphine (C₁₇H₁₉NO₅·HCl: 285.34), and not less than 35.0% and not more than 41.0% of other opium alkaloids.

Description Opium Alkaloids Hydrochlorides occur as a white to light brown powder.

It is soluble in water, and slightly soluble in ethanol (99.5).

It is colored by light.

Identification (1) Dissolve 0.1 g of Opium Alkaloids Hydrochlorides in 10 mL of diluted ethanol (1 in 2), and use this solution as the sample solution. Separately, dissolve 60 mg of Morphine Hydrochloride Hydrate, 40 mg of Noscapine Hydrochloride Hydrate, 10 mg of Codein Phosphate Hydrate and 10 mg of Papaverine Hydrochloride in 10 mL each of diluted ethanol (1 in 2), and use these solutions as the standard solutions (1), (2), (3) and (4), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography (5.03). Spot 20 μL each of the sample solution and standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene, ethanol (99.5) and ammonia solution (28:20:20:3:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): each spot from the sample solution and standard solutions (1), (2), (3) and (4) (morphine, noscapine, codeine and papaverine) should be the same in color tone and Rf value with the corresponding spot from the standard solutions (1), (2), (3) and (4).

(2) A solution of Opium Alkaloids Hydrochlorides (1 in 50) responds to the Qualitative Tests (5.09) (2) for chloride.

pH <2.54> Dissolve 1.0 g of Opium Alkaloids Hydrochlorides in 50 mL of water: the pH of the solution is between 3.0 and 4.0.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Opium Alkaloids Hydrochlorides in 10 mL of water: the solution is clear, and its absorbance <2.24> at 420 nm is not more than 0.20.

(2) Meconic acid—Dissolve 0.1 g of Opium Alkaloids Hydrochlorides in 2 mL of water, and pour into a polyethylene column 1 cm in inside diameter, packed with about 0.36 g of aminopropylsilanized silica gel for pretreatment (55 – 105 μm in particle diameter) and previously washed through with 5 mL of water. Then, wash the column with 5 mL of water, 5 mL of methanol and 10 mL of 0.1 mol/L hydrochloric acid in this order, then elute with 2 mL of 1 mol/L hydrochloric acid, and use the eluate as the test solution. To the test solution add 2 mL of dilute sodium hydroxide TS and 1 drop of iron (III) chloride TS: no red color develops.

Loss on drying <2.41> Not more than 6.0% (0.5 g, 120°C, 8 hours).

Residue on ignition <2.44> Not more than 0.5% (0.5 g).

Assay Weigh accurately about 0.1 g of Opium Alkaloids Hydrochlorides, and dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of morphine hydrochloride for assay, dissolve in water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine the peak areas of morphine, codeine, papaverine, thebaine, narceine and noscapine, A₁₁, A₁₂, A₁₃, A₁₄, A₁₅ and A₁₆, from the sample solution, and the peak area of morphine, Aₛ, from the standard solution.

Amount (mg) of morphine (C₁₇H₁₉NO₅·HCl)

\[ M₅ = \frac{A₁₇}{A₅} \times 0.887 \]

Amount (mg) of other opium alkaloids

\[ M₅ = \frac{A₁₇}{A₅} \times (0.29A₁₄ + 0.20A₁₃ + 0.19A₁₅ + A₁₁) \times 0.887 \]

Mₛ: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

The relative retention time of codeine, papaverine, thebaine, narceine and noscapine with respect to morphine obtained under the following operating conditions are as follows.

<table>
<thead>
<tr>
<th>Component</th>
<th>Relative retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>codeine</td>
<td>1.1</td>
</tr>
<tr>
<td>papaverine</td>
<td>1.9</td>
</tr>
<tr>
<td>thebaine</td>
<td>2.5</td>
</tr>
<tr>
<td>narceine</td>
<td>2.8</td>
</tr>
<tr>
<td>noscapine</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Operating conditions—


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecyilsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about
Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

System suitability—
System performance: Dissolve 60 mg of Morphine Hydrochloride Hydrate, 10 mg of Codeine Phosphate Hydrate, 10 mg of Papaverine Hydrochloride and 40 mg of Noscapine Hydrochloride Hydrate in water to make 50 mL. When the procedure is run with 20 µL of this solution under the above operating conditions, morphine, codeine, papaverine and noscapine are eluted in this order with the complete separation between these peaks and with the resolution between the peaks of morphine and codeine being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of morphine is not more than 1.0%.

Containers and storage — Containers—Tight containers.
Storage—Light-resistant.

Opium Alkaloids Hydrochlorides Injection

アヘンアルカロイド塩酸塩注射液

Opium Alkaloids Hydrochlorides Injection is an aqueous solution for injection.

It contains not less than 0.90 w/v% and not more than 1.10 w/v% of morphine (C17H19NO3: 285.34).

Method of preparation

| Opium Alkaloids Hydrochlorides | 20 g |
| Water for Injection or Sterile Water for Injection in Containers | a sufficient quantity |

Prepare as directed under Injections, with the above ingredients.

Description Opium Alkaloids Hydrochlorides Injection is a clear, colorless or light brown liquid.

It is affected by light.

pH: 2.5 – 3.5

Identification To 1 mL of Opium Alkaloids Hydrochlorides Injection add 1 mL of ethanol (99.5), mix, and use this solution as the sample solution, and proceed as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

Extractable volume <6.0> It meets the requirement.

Assay Pipet 2 mL of Opium Alkaloids Hydrochlorides Injection, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, and dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_T \) and \( Q_S \), of the peak area of morphine to that of the internal standard.

\[
\text{Amount (mg) of morphine (C}_{17}\text{H}_{19}\text{NO}_3) = M_S \times \frac{Q_T}{Q_S} \times 0.887
\]

\( M_S \): Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

System suitability—
System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

Containers and storage — Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.
Opium Alkaloids and Atropine Injection

アヘンアルカロイド・アトロピン注射液

Opium Alkaloids and Atropine Injection is an aqueous solution for injection. It contains not less than 0.90 w/v% and not more than 1.10 w/v% of morphine \((C_{17}H_{19}NO_3): 285.34\), and not less than 0.027 w/v% and not more than 0.033 w/v% of atropine sulfate hydrate \((C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O: 694.84\).

Method of preparation

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opium Alkaloids Hydrochlorides</td>
<td>20 g</td>
<td>For injection</td>
</tr>
<tr>
<td>Atropine Sulfate Hydrate</td>
<td>0.3 g</td>
<td>For solution analysis</td>
</tr>
<tr>
<td>Water for Injection or Sterile Water</td>
<td>a sufficient quantity</td>
<td>To make 1000 mL</td>
</tr>
</tbody>
</table>

Prepare as directed under Injections, with the above ingredients.

Description

Opium Alkaloids and Atropine Injection is a colorless or light brown, clear liquid. It is affected by light.

pH: 2.5 - 3.5

Identification

Identification (1) To 1 mL of Opium Alkaloids and Atropine Injection add 1 mL of ethanol (99.5%), mix, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

(2) To 2 mL of Opium Alkaloids and Atropine Injection add 2 mL of ammonia TS, extract with 10 mL of diethyl ether, and filter the diethyl ether layer. Evaporate the filtrate on a water bath to dryness, add 1 mL of ethanol (99.5) to the residue, and heat to dissolve. Allow to stand this solution in an ice water for 30 minutes with occasional shaking. After crystals are formed, use the supernatant liquid as the sample solution. Separately, dissolve 0.03 g of Atropine Sulfate RS in 100 mL of water, proceed with 2 mL of this solution in the same manner as for the sample solution, and use a solution so obtained as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(2.02\). Spot 10 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia water (28) (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff’s TS for spraying on the plate: a spot of about 0.2 Rf value among the several spots from the sample solution and an orange colored spot from the standard solution show the same color tone, and have the same Rf value (atropine).

Extractable volume \(\leq 5.0\) It meets the requirements.

Assay

(1) Morphine—Pipet 2 mL of Opium Alkaloids and Atropine Injection, add exactly 10 mL of the internal standard solution, then add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, then add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography \(2.01\) according to the following conditions, and calculate the ratios, \(Q_T\) and \(Q_S\), of the peak area of morphine to that of the internal standard.

\[
\text{Amount (mg) of morphine (C}_{17}\text{H}_{19}\text{NO}_{3}) = M_S \times \frac{Q_T}{Q_S} \times 0.887
\]

\(M_S\): Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of ethylefrine hydrochloride (1 in 500).

Operating conditions—


Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 \(\mu\)L of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 \(\mu\)L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 2.0%.

(2) Atropine sulfate hydrate—Pipet 2 mL of Opium Alkaloids and Atropine Injection, add exactly 2 mL of the internal standard solution, and add 10 mL of diluted dilute hydrochloric acid (1 in 10). Shake this solution with two 10-mL portions of dichloromethane. Remove the dichloromethane layer, to the water layer add 2 mL of ammonia TS, immediately add 20 mL of dichloromethane, shake vigorously, filter the dichloromethane extract through filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloromethane and 0.5 mL of bis-trimethylsilylacetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Atropine Sulfate RS (determine separately the loss on drying \(\leq 2.4\) under the same conditions as Atropine Sulfate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, and add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as directed for the sample solution, and use this solution as the standard solution. Perform the test with 2 \(\mu\)L each of the sample solution and standard solution as directed under Gas Chromatography \(2.02\) according to the following conditions, and calculate the ratios, \(Q_T\) and \(Q_S\), of the peak area of atropine to that of the internal standard.
the peak area of atropine to that of the internal standard.

Amount (mg) of atropine sulfate hydrate
\[
(C_{17}H_{23}NO_3)_{2} \cdot H_2SO_4 \cdot H_2O
\]

\[
M_2 = \frac{M_S \times Q_L/Q_S \times 1/50 \times 1.027}{1/50 \times 1.027}
\]

Internal standard solution—A solution of homatropine hydrobromide (1 in 4000).

Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A glass column 3 mm in inside diameter and 1.5 m in length, packed with 180 to 250 μm siliceous earth for gas chromatography coated in 1 to 3% with 50% phenylmethyl silicone polymer for gas chromatography.
Column temperature: A constant temperature of about 210°C.
Carrier gas: Nitrogen or helium.
Flow rate: Adjust the flow rate so that the retention time of atropine is about 5 minutes.
System suitability—
System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the internal standard and atropine are eluted in this order with the resolution between these peaks being not less than 3.
System repeatability: When the test is repeated 5 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of atropine to that of the internal standard is not more than 2.0%.

Containers and storage—Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

Opium Alkaloids and Scopolamine Injection
アヘンアルカロイド・スコポラミン注射液

Opium Alkaloids and Scopolamine Injection is an aqueous solution for injection.

It contains not less than 1.80 w/v% and not more than 2.20 w/v% of morphine (C17H19NO3: 285.34) and not less than 0.054 w/v% and not more than 0.066 w/v% of scopolamine hydrobromide hydrate (C17H23NO3.HBr.3H2O: 438.31).

Method of preparation

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opium Alkaloids Hydrochlorides</td>
<td>40</td>
</tr>
<tr>
<td>Scopolamine Hydrobromide Hydrate</td>
<td>0.6</td>
</tr>
<tr>
<td>Water for Injection or Sterile Water</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

Description—Opium Alkaloids and Scopolamine Injection is a clear, colorless to light brown liquid.

It is affected by light.

pH: 2.5 – 3.5

Identification (1) To 1 mL of Opium Alkaloids and Scopolamine Injection add 1 mL of water and 2 mL of ethanol (99.5), mix, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

(2) To 1 mL of Opium Alkaloids and Scopolamine Injection add 1 mL of water and 2 mL of ammonia TS, extract with 10 mL of diethyl ether, and filter the diethyl ether layer. Evaporate the filtrate on a water bath to dryness, add 1 mL of ethanol (99.5) to the residue, and heat to dissolve. Allow to stand this solution in an ice water for 30 minutes with occasional shaking. After crystals are formed, use the supernatant liquid as the sample solution. Separately, dissolve 0.03 g of Scopolamine Hydrobromide RS in 100 mL of water. To 2 mL of this solution add 2 mL of ammonia TS, proceed with this solution in the same manner as for the sample solution, and use a solution so obtained as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>.

Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia water (28) (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff’s TS for spraying on the plate: a spot of about 0.7 Rf value among the several spots from the sample solution and an orange colored spot from the standard solution show the same color tone, and have the same Rf value (scopolamine).

Extractable volume <6.05> It meets the requirements.

Assay (1) Morphine—Pipet 1 mL of Opium Alkaloids and Scopolamine Injection, add 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.03> according to the following conditions, and calculate the ratios, \( Q_L / Q_S \), of the peak area of morphine to that of the internal standard.

\[
M_5 = \frac{M_S \times Q_L/Q_S \times 0.887}{M_S \times Q_L/Q_S \times 0.887}
\]

Internal standard solution—A solution of etilefrin hydrochloride (1 in 500).

Operating conditions—
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.
Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

**System suitability**—
System performance: When the procedure is run with 20 \( \mu \text{L} \) of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 \( \mu \text{L} \) of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 2.0%.

(2) Scopolamine hydrobromide hydrate—Pipet 2 \( \text{mL} \) of Opium Alkaloids and Scopolamine Injection, and add exactly 2 \( \text{mL} \) of the internal standard solution. To this solution add 10 \( \text{mL} \) of diluted hydrochloric acid (1 in 10), and shake with two 10-\( \text{mL} \) portions of dichloromethane. Remove the dichloromethane layer to the water layer add 2 \( \text{mL} \) of ammonia \( \text{TS} \), add immediately 20 \( \text{mL} \) of dichloromethane, shake vigorously, filter the dichloromethane extract through a filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 \( \text{mL} \) of 1,2-dichloroethane and 0.5 \( \text{mL} \) of bis-trimethyl silyl acetamide, stopper tightly, warm in a water bath at 60\( ^\circ \)C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of Scoponamine Hydrobromide RS (determine separately the loss on drying \( \text{C}_17\text{H}_{21}\text{NO}_4\cdot\text{HBr} \cdot 3\text{H}_2\text{O} \)) and add exactly 2 \( \text{mL} \) of this solution in the same manner as for the sample solution, and use this solution as the standard solution. Perform the test with 2 \( \mu \text{L} \) each of the sample solution and standard solution as directed under Gas Chromatography \( \text{C}_17\text{H}_{21}\text{NO}_4\cdot\text{HBr} \cdot 3\text{H}_2\text{O} \). and calculate the ratios, \( Q_t \) and \( Q_s \), of the peak area of scopolamine to that of the internal standard.

\[
\text{Amount (mg) of scopolamine hydrobromide hydrate (C}_17\text{H}_{21}\text{NO}_4\cdot\text{HBr} \cdot 3\text{H}_2\text{O}) = M_s \times Q_t/Q_s \times 1/50 \times 1.141
\]

\( M_s \): Amount (mg) of Scopolamine Hydrobromide RS, calculated on the dried basis.

**Internal standard solution**—A solution of homatropine hydrobromide (1 in 4000).

**Operating conditions**—
Detector: A hydrogen flame-ionization detector.
Column: A glass column 3 mm in inside diameter and 1.5 m in length, packed with 180 to 250 g of siliceous earth for gas chromatography coated in 1 to 3% with 50% phenylmethyl silicone polymer for gas chromatography.
Column temperature: A constant temperature of about 210\( ^\circ \)C.
Carrier gas: Nitrogen or helium.
Flow rate: Adjust the flow rate so that the retention time of scopolamine is about 8 minutes.

**System suitability**—
System performance: When the procedure is run with 2 \( \mu \text{L} \) of the standard solution under the above operating conditions, the internal standard and scopolamine are eluted in this order with the resolution between these peaks being not less than 6.
System repeatability: When the test is repeated 5 times with 2 \( \mu \text{L} \) of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of scopolamine to that of the internal standard is not more than 2.0%.

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

**Weak Opium Alkaloids and Scopolamine Injection**
弱アヘンアルカロイド・スコポラミン注射液

Weak Opium Alkaloids and Scopolamine Injection is an aqueous solution for injection.

It contains not less than 0.90 \% and not more than 1.10 \% of morphine \((\text{C}_17\text{H}_{19}\text{NO}_3)\) and not less than 0.027 \% and not more than 0.033 \% of scopolamine hydrobromide hydrate \((\text{C}_17\text{H}_{21}\text{NO}_4\cdot\text{HBr} \cdot 3\text{H}_2\text{O})\).

**Method of preparation**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opium Alkaloids Hydrochlorides</td>
<td>20 g</td>
</tr>
<tr>
<td>Scopolamine Hydrobromide Hydrate</td>
<td>0.3 g</td>
</tr>
</tbody>
</table>

Prepare as directed under Injections, with the above ingredients.

**Description** Weak Opium Alkaloids and Scopolamine Injection is a clear, colorless or light brown liquid.

It is affected by light.

pH: 2.5 - 3.5

**Identification** (1) To 1 \( \text{mL} \) of Opium Alkaloids and Scopolamine Injection add 1 \( \text{mL} \) of ethanol (99.5%), mix, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

(2) To 2 \( \text{mL} \) of Weak Opium Alkaloids and Scopolamine Injection add 2 \( \text{mL} \) of ammonia \( \text{TS} \), extract with 10 \( \text{mL} \) of diethyl ether, and filter the diethyl ether layer. Evaporate the filtrate on a water bath to dryness, add 1 \( \text{mL} \) of ethanol (99.5) to the residue, and heat to dissolve. Allow to stand this solution in an ice water for 30 minutes with occasional shaking. After crystals are formed, use the supernatant liquid as the sample solution. Separately, dissolve 0.03 g of Scopolamine Hydrobromide RS in 100 \( \text{mL} \) of water, proceed with 2 \( \mu \text{L} \) of this solution in the same manner as for the sample solution, and use a solution so obtained as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( \text{C}_17\text{H}_{21}\text{NO}_4\cdot\text{HBr} \cdot 3\text{H}_2\text{O} \). Spot 10 \( \mu \text{L} \) each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia water (28)
Orange Oil / Official Monographs

**Extractable volume**

It meets the requirements.

**Assay (1)** Morphine—pipet 2 mL of Weak Opium Alkaloids and Scopolamine Injection, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL of the internal standard and sample solution as directed under Liquid Chromatography <2.02> according to the following conditions, and calculate the ratios, QT and QS, of the peak area of morphine to that of the internal standard.

\[
\text{Amount (mg) of morphine (C}_{17}\text{H}_{19}\text{NO}_{3}) = M_5 \times \frac{Q_T}{Q_S} \times 0.887
\]

**Internal standard solution**—A solution of etilefrin hydrochloride (1 in 500).

**Operating conditions**—

- **Detector:** An ultraviolet absorption photometer (wavelength: 285 nm).
- **Column:** A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- **Column temperature:** A constant temperature of about 40°C.
- **Mobile phase:** Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.
- **Flow rate:** Adjust the flow rate so that the retention time of morphine is about 10 minutes.

**System suitability**—

- **System performance:** When the procedure is run with 20 μL of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

**System repeatability:** When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 2.0%.

**Extractable volume**

Under reduced pressure. To the residue add 0.5 mL of 1,2-dichloroethane and 0.5 mL of bis-trimethylsilyl acetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of Scopolamine Hydrobromide RS (separately determine the loss on drying <2.41> under the same conditions as Scopolamine Hydrobromide Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as for the sample solution, and use so obtained solution as the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, QT and QS, of the peak area of scopolamine to that of the internal standard.

\[
\text{Amount (mg) of scopolamine hydrobromide hydrate (C}_{17}\text{H}_{21}\text{NO}_{4}.\text{HBr.}3\text{H}_{2}\text{O}) = M_5 \times \frac{Q_T}{Q_S} \times 1/50 \times 1.141
\]

**Containers and storage**—Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

**Orange Oil**

**Oleum Aurantii**

オレンジ油

Orange Oil is the essential oil obtained by expression from the peel of the edible fruit of *Citrus* species (*Rutaceae*).

**Description**—Orange Oil is a yellow to yellow-brown liquid.
Orciprenaline Sulfate

オルシプレナリン硫酸塩

Orciprenaline Sulfate occurs as white crystals or crystalline powder.

It is freely soluble in water, slightly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

Description

A solution of Orciprenaline Sulfate (1 in 20) shows no optical rotation.

Melting point: about 220°C (with decomposition).

Identification (1) Determine the determination spectrum of a solution of Orciprenaline Sulfate in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Orciprenaline Sulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Orciprenaline Sulfate (1 in 100) responds to the Qualitative Tests <1.09> for sulfate.

pH <2.54> Dissolve 1.0 g of Orciprenaline Sulfate in 10 mL of water: the pH of this solution is between 4.0 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Orciprenaline Sulfate in 10 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To 3 mL of Matching Fluid T add 1 mL of diluted hydrochloric acid (1 in 40).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Orciprenaline Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Orciprenalone—Dissolve 0.200 g of Orciprenaline Sulfate in 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance at 328 nm is not more than 0.075.

Loss on drying <2.4> Not more than 1.5% (1 g, in vacuum, 105°C, 4 hours).

Residue on ignition <2.4> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Orciprenaline Sulfate, dissolve in 100 mL of acetic acid (100) by warming on a water bath, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 52.06 mg of \( (C_{11}H_{17}NO_3)_2H_2SO_4 \).

Oxapinum Iodide

オキサピウムヨウ化物

Oxapinum Iodide occurs as a white, crystalline powder.

It is soluble in acetonitrile, in methanol and in ethanol (95), slightly soluble in water, in acetic anhydride and in acetic acid (100), and practically insoluble in diethyl ether.

A solution of Oxapinum Iodide according to Method 2, and perform the test. Prepare the control solution with 2.0 g of Oxapinum Iodide, previously dried, as directed under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Identification (1) Determine the infrared absorption spectrum of Oxapinum Iodide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 0.1 g of Oxapinum Iodide in 10 mL of methanol, and add 2 mL of dilute nitric acid and 2 mL of silver nitrate solution.
Weigh accurately about 0.7 g of Oxapium Iodide, and indicate the residue on ignition.

Standard solution composes 5 to 15 mg of Oxapium from the standard solution. The sample solution is not larger than the area of the peak of Oxapium from the standard solution.

The following conditions. Determine each peak area of each solution by the automatic integration method: the total area of the peaks other than the peak of oxapium from the sample solution is not larger than the area of the peak of oxapium from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of 20°C to 30°C.

Mobile phase: To 57 mL of acetic acid (100) and 139 mL of triethylamine add water to make 1000 mL. To 50 mL of this solution add 500 mL of acetonitrile, 10 mL of dilute acetic acid and 440 mL of water.

Flow rate: Adjust the flow rate so that the retention time of oxapium is about 4 minutes.

Selection of column: Dissolve 0.05 g of Oxapium Iodide and 3 mg of benzophenone in 100 mL of the mobile phase. Proceed with 20 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of oxapium and benzophenone in this order with the resolution between these peaks being not less than 5.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of oxapium obtained from 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of each solution by the automatic integration method: the total area of the peaks other than the peak of oxapium from the sample solution is not larger than the area of the peak of oxapium from the standard solution.

Melting point <2.60> 198 – 203°C.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Oxapium Iodide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.05 g of Oxapium Iodide in 100 mL of a mixture of water and acetonitrile (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and acetonitrile (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of each solution by the automatic integration method: the total area of the peaks other than the peak of oxapium from the sample solution is not larger than the area of the peak of oxapium from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Oxapium Iodide, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (9:1), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, platinum electrode). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 47.14 mg of C21H15INO3.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Oxapium

オキサプロジン

C18H15NO3; 293.32
3-(4,5-Diphenyloxazol-2-yl)propanoic acid [21256-18-8]

Oxaprozin, when dried, contains not less than 98.5% of C18H15NO3.

Description Oxaprozin occurs as a white to yellowish white crystalline powder.

It is sparingly soluble in methanol and in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

It is gradually affected by light.

Identification Determine the infrared absorption spectrum of Oxaprozin, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance <2.24> E1%1cm (285 nm): 455 – 495 (after drying, 10 mg, methanol, 1000 mL).

Melting point <2.60> 161 – 165°C.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Oxaprozin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.17>—Prepare the test solution with 2.0 g of Oxaprozin according to Method 3, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 0.10 g of Oxaprozin in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 5 mL, 3 mL and 1 mL of this solution, add methanol to each to make exactly 10 mL, and use these solutions as the standard solutions (2), (3) and (4), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 10 μL each of the sample solution and standard solutions (1), (2), (3) and (4) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and acetic acid (100) (99:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the total intensity of the spots other than the principal spot from the sample solution is not more than 1.0% calculated on the basis of intensities of the spots from the standard solutions (1), (2), (3) and (4).

Loss on drying <2.41> Not more than 0.3% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.3% (1 g).
Oxazolam occurs as white crystals or crystalline powder.

It is odorless and tasteless.

It is freely soluble in acetic acid (100), soluble in 1,4-dioxane and in dichloromethane, slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

It gradually changes in color by light.

Melting point: about 187°C (with decomposition).

Identification (1) Dissolve 0.01 g of Oxazolam in 5 mL of dilute hydrochloric acid by heating in a water bath for 10 minutes. After cooling, 1 mL of this solution responds to the Qualitative Tests <1,00> for primary aromatic amines.

(3) Place 2 g of Oxazolam in a 200-mL flask, add 50 mL of ethanol (95) and 25 mL of 6 mol/L hydrochloric acid TS, and boil under a reflux condenser for 5 hours. After cooling, neutralize with a solution of sodium hydroxide (1 in 4), and extract with 30 mL of dichloromethane. Dehydrate with 3 g of anhydrous sodium sulfate, filter, and evaporate the dichloromethane of the filtrate. Dissolve the residue in 20 mL of methanol by heating on a water bath, and cool immediately in an ice bath. Collect the crystals, and dry in vacuum at 60°C for 1 hour: the crystals melt <2,59> between 96°C and 100°C.

(4) Determine the absorption spectrum of a solution of Oxazolam in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2,247> and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) Proceed with Oxazolam as directed under Flame Coloration Test <1,04> (2), and perform the test: a green color appears.

Absorbance <2,247> $E_{1\%}^{1\text{cm}}$ (246 nm): 410 – 430 (after drying, 1 mg, ethanol (95), 100 mL).

Purity (1) Chloride <1,03>—To 1.0 g of Oxazolam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. To 25 mL of this filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2) Heavy metals <1,07>—Proceed with 1.0 g of Oxazolam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1,11>—Place 1.0 g of Oxazolam in a Kjeldahl flask, add 5 mL of sulfuric acid and 5 mL of nitric acid, and heat gently. Repeat the addition of 2 to 3 mL of nitric acid at times, and continue to heat until a colorless to light yellow solution is obtained. After cooling, add 15 mL of saturated ammonium oxalate monohydrate solution, heat the solution until dense white fumes are evolved, and evaporate to a volume of 2 to 3 mL. After cooling, dilute with water to 10 mL, and perform the test with this solution as the test solution (not more than 2 ppm).

(4) Related substances—Dissolve 0.05 g of Oxazolam in 10 mL of dichloromethane, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dichloromethane to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2,037>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Immediately air-dry, develop the plate with a mixture of toluene and acetone (8:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2,41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2,44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.65 g of Oxazolam, previously dried, dissolve in 100 mL of a mixture of acetic acid (100) and 1,4-dioxane (1:1). Titrate <2,59> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 32.88 mg of C$_{18}$H$_{17}$ClN$_2$O$_2$. 

Containers and storage Containers—Tight containers. Storage—Light-resistant.
Oxethazaine

Oxetacaine

Oキセサゼイン

C₂₈H₄₁N₃O₃: 467.64
2,2'-2-Hydroxyethylimino)bis[N-(1,1-dimethyl-2-phenylethyl)-N-methylacetamide]
[126-27-2]

Oxethazaine, when dried, contains not less than 98.5% of C₂₈H₄₁N₃O₃.

Description Oxethazaine occurs as a white to pale yellowish white, crystalline powder.

Identification (1) Determine the absorption spectrum of a solution of Oxethazaine in ethanol (95) (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

2 Determine the infrared absorption spectrum of Oxethazaine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 101 - 104°C.

Purity (1) Chloride <1.07>—Dissolve 1.0 g of Oxethazaine in 20 mL of ethanol (95), add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS, 20 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.011%).

2 Heavy metals <1.07>—Proceed with 2.0 g of Oxethazaine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.40 g of Oxethazaine in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.63>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of isopropanol, tetrahydrofuran, methanol and ammonia solution (28:24:10:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

4 2-Aminoethanol—To 1.0 g of Oxethazaine add methanol to make exactly 10 mL, then add 0.1 mL of a solution of 1-fluoro-2,4-dinitrobenzene in methanol (1 in 25), shake well, and heat at 60°C for 20 minutes: the solution has no more color than the following control solution.

Control solution: To 0.10 g of 2-aminoethanol add methanol to make exactly 200 mL, pipet 1 mL of this solution, and add methanol to make exactly 10 mL. Proceed as directed above.

Loss on drying <2.47> Not more than 0.5% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.9 g of Oxethazaine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 46.76 mg of C₂₈H₄₁N₃O₃

Containers and storage Containers—Tight containers.

Oxprenolol Hydrochloride

オクスプレノロール塩酸塩

C₁₅H₂₃NO₃.HCl: 301.81
(2RS)-1-[2-(Allyloxy)phenoxyl]-3-(1-methylethyl)aminopropan-2-ol monohydrochloride [6452-73-9]

Oxprenolol Hydrochloride, when dried, contains not less than 98.5% of C₁₅H₂₃NO₃.HCl.

Description Oxprenolol Hydrochloride occurs as a white, crystalline powder.

It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Identification (1) To 2 mL of a solution of Oxprenolol Hydrochloride (1 in 100) add 1 drop of copper (II) sulfate TS and 2 mL of sodium hydroxide TS: a blue-purple color develops. To this solution add 1 mL of diethyl ether, shake well, and allow to stand: a red-purple color develops in the diethyl ether layer, and a blue-purple color develops in the water layer.

2 To 3 mL of a solution of Oxprenolol Hydrochloride (1 in 150) add 3 drops of Reinecke salt TS: a light red precipitate is formed.

3 Determine the infrared absorption spectrum of Oxprenolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

4 A solution of Oxprenolol Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.
Oxybuprocaine Hydrochloride

Benoxinate Hydrochloride

オキシブプロカイン塩酸塩

C₁₇H₂₈N₂O₃·HCl: 344.88
2-(Diethylamino)ethyl 4-amino-3-butyloxybenzoate monohydrochloride

[5987-82-6]

Oxybuprocaine Hydrochloride, when dried, contains not less than 99.0% of C₁₇H₂₈N₂O₃·HCl.

Description Oxybuprocaine Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a saline taste. It exhibits anesthetic properties when placed on the tongue.

It is very soluble in water, freely soluble in ethanol (95%) and in chloroform, and practically insoluble in diethyl ether.

The pH of a solution of Oxybuprocaine Hydrochloride (1 in 10) is between 5.0 and 6.0.

It is gradually colored by light.

Identification (1) Dissolve 0.01 g of Oxybuprocaine Hydrochloride in 1 mL of dilute hydrochloric acid and 4 mL of water. This solution responds to the Qualitative Tests for primary aromatic amines.

(2) Dissolve 0.1 g of Oxybuprocaine Hydrochloride in 8 mL of water, and add 3 mL of ammonium thiocyanate TS: an oily substance is produced. Rub the inner surface of the container with a glass rod: white crystals are formed. Collect the crystals so obtained, recrystallize from water, and dry in a desiccator (in vacuum, phosphorus (V) oxide) for 5 hours: the crystals melt between 103°C and 106°C.

(3) Determine the absorption spectrum of a solution of Oxybuprocaine Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution of Oxybuprocaine Hydrochloride (1 in 10) responds to the Qualitative Tests for chloride.

Melting point 158–162°C.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Oxybuprocaine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals—Proceed with 1.0 g of Oxybuprocaine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.25 g of Oxybuprocaine Hydrochloride in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet 1 mL of this solution, add chloroform to make exactly 20 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography.

Melting point 107–110°C.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Oxprenolol Hydrochloride in 10 mL of water: the pH of this solution is between 4.5 and 6.0.

(2) Heavy metals—Proceed with 2.0 g of Oxprenolol Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Oxprenolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.25 g of Oxprenolol Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 4 mL of the sample solution, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography.

Loss on drying Not more than 0.5% (1 g, 80°C, 3 hours).

Residue on ignition Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Oxprenolol Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 30.18 mg of C₁₇H₂₄N₂O₃·HCl

Containers and storage Containers—Tight containers.
Weigh accurately about 0.6 g of Oxybuprocaine Hydrochloride Hydrate. Solution is clear and colorless. The pH of a solution dissolved 1.0 g of Oxycodone Hydrochloride Hydrate in 10 mL of water: it is between 3.8 and 5.8.

Place the plate in the rotary evaporator, and for 2 hours.

Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Oxybuprocaine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 34.49 mg of C17H28N2O3.HCl

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Oxycodone Hydrochloride Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Morphine—Dissolve 10 mg of Oxycodone Hydrochloride Hydrate in 1 mL of water, add 5 mL of 1-nitroso-2-naphthol (III) (1 in 100), and shake. Then shake the solution with 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. To this solution add 1 mL of a solution of sodium nitrite (1 in 5000), and warm at 40°C for 5 minutes. After cooling, add 10 mL of chloroform, shake, centrifuge, and separate the water layer: the color of the solution is not more intense than a pale red.

(3) Codeine—Dissolve 10 mg of Oxycodone Hydrochloride Hydrate in 5 mL of sulfuric acid, add 1 drop of iron (III) chloride TS, and warm: no blue color is produced. Add 1 drop of nitric acid: no red color develops.

(4) Thebaine—Dissolve 0.10 g of Oxycodone Hydrochloride Hydrate in 2 mL of diluted hydrochloric acid (1 in 10), and heat the solution in a water bath for 25 minutes. After cooling, add 0.5 mL of 4-aminoantipyrine hydrochloride TS and 0.5 mL of a solution of potassium hexacyanoferrate (III) (1 in 100), and shake. Then shake the solution with 2 mL of ammonia TS and 3 mL of chloroform: no red color develops in the chloroform layer.

Water 12 – 15% (0.2 g, volumetric titration, direct titration).

Residue on ignition Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.5 g of Oxycodone Hydrochloride Hydrate, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 35.18 mg of C18H21NO4.HCl
**Compound Oxycodone and Atropine Injection**

**Compound Oxycodone Injection**

**Compound Hycodenone Injection**

複方オキシコドン注射液

Compound Oxycodone Injection is an aqueous solution for injection.

It contains not less than 0.74 w/v% and not more than 0.86 w/v% of oxycodone hydrochloride hydrate (C₁₈H₂₁NO₄.HCl.H₂O: 405.87), and not less than 0.18 w/v% and not more than 0.22 w/v% of hydrocotarnine hydrochloride hydrate (C₁₂H₁₅NO₃.HCl.H₂O: 275.73).

### Method of preparation

<table>
<thead>
<tr>
<th>Compound Hydrochloride Hydrate</th>
<th>8 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocotarnine Hydrochloride Hydrate</td>
<td>2 g</td>
</tr>
<tr>
<td>Water for Injection or Sterile Water</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

Prepare as directed under Injections, with the above ingredients.

**Description** Compound Oxycodone Injection is a clear, colorless to pale yellow liquid.

It is affected by light.

pH: 2.5 – 4.0

**Identification** (1) To 1 mL of Compound Oxycodone Injection add 1 mL of 2,4-dinitrophenylhydrazine-ethanol TS: a yellow precipitate is formed (oxycodone).

(2) Evaporate 1 mL of Compound Oxycodone Injection on a water bath. Dissolve the residue in 2 mL of sulfuric acid: a yellow color is produced. Heat the solution: it changes to red, and then to deep orange-red (hydrocotarnine).

(3) Evaporate 1 mL of Compound Oxycodone Injection on a water bath. Dissolve the residue in 3 mL of sulfuric acid, add 2 drops of a solution of tannic acid in ethanol (95) (1 in 20), and allow to stand: a deep green color is produced (hydrocotarnine).

**Extractable volume** <6.05> It meets the requirement.

**Assay** Pipet 2 mL of Compound Oxycodone Injection, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of oxycodone hydrochloride for assay and about 0.1 g of hydrocotarnine hydrochloride for assay previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Calculate the ratios, Qₐ and Qₙb, of the peak area of oxycodone and hydrocotarnine to that of the internal standard from the sample solution, and the ratios, Qₙa and Qₙb, of the peak area of oxycodone and hydrocotarnine to that of the internal standard from the standard solution.

Amount (mg) of oxycodone hydrochloride hydrate (C₁₈H₂₁NO₄.HCl.H₂O): \[ \frac{M_{SH} \times Q_{Sb}}{Q_{Sa}} \times \frac{1}{25} \times 1.154 \]

Amount (mg) of hydrocotarnine hydrochloride hydrate (C₁₂H₁₅NO₃.HCl.H₂O): \[ \frac{M_{SH} \times Q_{Sb}}{Q_{Sa}} \times \frac{1}{25} \times 1.070 \]

**Compound Oxycodone and Atropine Injection**

**Hycoato Injection**

複方オキシコドン・アトロピン注射液

Compound Oxycodone and Atropine Injection is an aqueous solution for injection.

It contains not less than 0.74 w/v% and not more than 0.86 w/v% of oxycodone hydrochloride hydrate (C₁₈H₂₁NO₄.HCl.H₂O: 405.87), not less than 0.18 w/v% and not more than 0.22 w/v% of hydrocotarnine hydrochloride hydrate (C₁₂H₁₅NO₃.HCl.H₂O: 275.73), and not less than 0.027 w/v% of atropine sulfate hydrate [(C₁₇H₂₃NO₃)₂.H₂SO₄.H₂O: 694.83].

Internal standard solution—Dissolve 0.02 g of phenacetin in 10 mL of ethanol (95), and add water to make 100 mL.

**Operating conditions**—


Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized polyvinyl alcohol gel polymer for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 500 mL of 0.05 mol/L disodium hydrogen phosphate TS add 0.05 mol/L sodium dihydrogen phosphate TS, and adjust the pH to 8.0. To 300 mL of this solution add 200 mL of acetonitrile, and mix.

Flow rate: Adjust the flow rate so that the retention time of oxycodone is about 8 minutes.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions, and use a column giving elution of the internal standard, oxycodone and hydrocotarnine in this order, with complete separation of these peaks.

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

**Compound Oxycodone and Atropine Injection**

**Hycoato Injection**

複方オキシコドン・アトロピン注射液

Compound Oxycodone and Atropine Injection is an aqueous solution for injection.

It contains not less than 0.74 w/v% and not more than 0.86 w/v% of oxycodone hydrochloride hydrate (C₁₈H₂₁NO₄.HCl.H₂O: 405.87), not less than 0.18 w/v% and not more than 0.22 w/v% of hydrocotarnine hydrochloride hydrate (C₁₂H₁₅NO₃.HCl.H₂O: 275.73), and not less than 0.027 w/v% of atropine sulfate hydrate [(C₁₇H₂₃NO₃)₂.H₂SO₄.H₂O: 694.83].
Method of preparation

<table>
<thead>
<tr>
<th>Compound</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxycodone Hydrochloride Hydrate</td>
<td>8 g</td>
</tr>
<tr>
<td>Hydrocotamine Hydrochloride Hydrate</td>
<td>2 g</td>
</tr>
<tr>
<td>Atropine Sulfate Hydrate</td>
<td>0.3 g</td>
</tr>
<tr>
<td>Water for Injection or Sterile Water</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

Prepare as directed under Injections, with the above ingredients.

Description

Compound Oxycodone and Atropine Injection is a colorless or pale yellow, clear liquid. It is affected by light.

pH: 2.5 - 4.0

Identification

(1) To 1 mL of Compound Oxycodone and Atropine Injection add 1 mL of 2,4-dinitrophenylhydrazine-ethanol TS: a yellow precipitate is formed (oxycodone).

(2) Evaporate 1 mL of Compound Oxycodone and Atropine Injection on a water bath, and dissolve the residue in 2 mL of sulfuric acid: a yellow color is produced. Heat the solution: it changes to red, and then to deep orange-red (hydrocotamine).

(3) Evaporate 1 mL of Compound Oxycodone and Atropine Injection on a water bath. Dissolve the residue in 3 mL of sulfuric acid, add 2 drops of a solution of tannic acid in ethanol (95) (1 in 20), and allow to stand: a deep green color is produced (hydrocotamine).

(4) To 1 mL of Compound Oxycodone and Atropine Injection add 0.5 mL of 2,4-dinitrophenylhydrazine-ethanol TS, and allow to stand for 1 hour. Centrifuge, and add acetone to the supernatant liquid until no more precipitate is produced. Allow to stand for 20 minutes, and centrifuge. To the supernatant liquid add potassium hydroxide TS until the solution is light purple. Shake the liquid with 5 mL of dichloromethane, and separate the dichloromethane layer. Take 0.5 mL of the dichloromethane layer, and evaporate to dryness on a water bath. Add 5 drops of fuming nitric acid to the residue, and evaporate to dryness on a water bath. Cool, dissolve the residue in 1 mL of N,N-dimethyformamide, and add 6 drops of tetraethylammonium hydroxide TS: a red-purple color is produced (atropine).

Extractable volume $< 6.05$ It meets the requirement.

Assay

(1) Oxycodone hydrochloride hydrate and hydrocotamine hydrochloride hydrate—Pipet 2 mL of Compound Oxycodone and Atropine Injection, and add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of oxycodone hydrochloride for assay and about 0.1 g of hydrocotamine hydrochloride for assay previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography $2.01$ according to the following conditions. Calculate the ratios, $Q_{S_b}$ and $Q_{S_a}$, of the peak area of oxycodone and hydrocotamine to that of the internal standard from the sample solution, and the ratios, $Q_{S_a}$ and $Q_{S_b}$, of the peak area of oxycodone and hydrocotamine to that of the internal standard from the standard solution.

\[
\text{Amount (mg) of oxycodone hydrochloride hydrate} = M_{S_b} \times Q_{S_a} / Q_{S_b} \times 1 / 25 \times 1.154
\]

\[
\text{Amount (mg) of hydrocotamine hydrochloride hydrate} = M_{S_b} \times Q_{S_0} / Q_{S_b} \times 1 / 25 \times 1.070
\]

$M_{S_b}$: Amount (mg) of oxycodone hydrochloride for assay, calculated on the anhydrous basis

$M_{S_b}$: Amount (mg) of hydrocotamine hydrochloride for assay

Internal standard solution—Dissolve 0.02 g of phenacetin in 10 mL of ethanol (95), and add water to make 100 mL.

Operating conditions—


Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized polyvinyl alcohol gel polymer for liquid chromatography ($5 \mu m$ in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 500 mL of 0.05 mol/L disodium hydrogenphosphate TS add 0.05 mol/L sodium dihydrogenphosphate TS, and adjust the pH to 8.0. To 300 mL of this solution add 200 mL of acetonitrile, and mix.

Flow rate: Adjust the flow rate so that the retention time of oxycodone hydrochloride is about 8 minutes.

Selection of column: Proceed with 10 µL of the standard solution under the above operating conditions, and use a column giving elution of the internal standard, oxycodone and hydrocotamine in this order with complete separation of these peaks.

(2) Atropine sulfate hydrate—Pipet 2 mL of Compound Oxycodone and Atropine Injection, and add exactly 2 mL of the internal standard solution. To this solution add 10 mL of diluted dilute hydrochloric acid (1 in 10) and 2 mL of ammonia TS, immediately add 20 mL of dichloromethane, shake vigorously, filter the dichloromethane layer through filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloromethane and 0.5 mL of bis-trimethylsilylacetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Atropine Sulfate RS (separately determine the loss on drying 2.02 according to the same conditions as Atropine Sulfate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, and add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as directed for the sample solution, and use so obtained solution as the standard solution. Perform the test with 2 µL each of the sample solution and standard solution as directed under Gas Chromatography $2.02$ according to the following conditions, and calculate the ratios, $Q_{T}$ and $Q_{S_b}$, of the peak area of atropine to that of the internal standards.

\[
\text{Amount (mg) of atropine sulfate hydrate} = M_S \times Q_{T} / Q_{S_b} \times 1 / 50 \times 1.027
\]

$M_S$: Amount (mg) of Atropine Sulfate RS, calculated on
Oxydol

オキシドール

Oxydol contains not less than 2.5 w/v% and not more than 3.5 w/v% of hydrogen peroxide (H₂O₂: 34.01). It contains suitable stabilizers.

Description Oxydol occurs as a clear, colorless liquid. It is odorless or has an odor resembling that of ozone.

It gradually decomposes upon standing or upon vigorous agitation.

It rapidly decomposes when in contact with oxidizing substances as well as reducing substances.

It, when alkalized, decomposes with effervescence.

It is affected by light.

pH: 3.0 - 5.0

Specific gravity d₂₀/₅: about 1.01

Identification 1 mL of Oxydol responds to the Qualitative Tests <1.09> for peroxide.

Purity (1) Acidity—To 25.0 mL of Oxydol add 2 drops of phenolphthalein TS and 2.5 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(2) Heavy metals <1.07>—To 5.0 mL of Oxydol add 20 mL of water and 2 mL of ammonia TS, evaporate on a water bath to dryness, dissolve the residue in 2 mL of dilute acetic acid by heating, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2 mL of dilute acetic acid, 2.5 mL of Standard Lead Solution and water to make 50 mL (not more than 5 ppm).

(3) Arsenic <1.11>—To 1.0 mL of Oxydol add 1 mL of ammonia TS, evaporate on a water bath to dryness, take the residue, prepare the test solution according to Method 1, and perform the test (not more than 2 ppm).

(4) Organic stabilizer—Extract 100 mL of Oxydol with 50-mL, 25-mL and 25-mL portions of a mixture of chloroform and diethyl ether (3:2) successively, combine the extracts in a tared vessel, and evaporate the combined extract on a water bath. Dry the residue over silica gel to constant mass: the mass of the residue is not more than 50 mg.

(5) Nonvolatile residue—Evaporate 20.0 mL of Oxydol on a water bath to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 20 mg.

Assay Pipet 1.0 mL of Oxydol, transfer it to a flask containing 10 mL of water and 10 mL of dilute sulfuric acid, and titrate 2.50 mL of 0.02 mol/L potassium permanganate VS.

Each mL of 0.02 mol/L potassium permanganate VS = 1.701 mg of H₂O₂

Containers and storage Containers—Tight containers. Storage—Light-resistant, and not exceeding 30°C.

Oxygen

酸素

O₂: 32.00

Oxygen is oxygen produced by the air liquification separation method.

It contains not less than 99.5 v/v% of O₂.

Description Oxygen is a colorless gas under atmospheric pressure, and is odorless.

1 mL of Oxygen dissolves in 32 mL of water, and in 7 mL of ethanol (95) at 20°C and at a pressure of 101.3 kPa.

1000 mL of Oxygen at 0°C and at a pressure of 101.3 kPa weighs 1.429 g.

Identification Transfer 1 mL each of Oxygen and oxygen directly from cylinders with a pressure-reducing valve to gas-measuring tubes or syringes for gas chromatography, using a polyvinyl chloride induction tube. Perform the test with these gases as directed under Gas Chromatography <2.02> according to the following conditions: the retention time of principal peak obtained from Oxygen is the same as that of the peak obtained from oxygen.

Operating conditions—

Proceed as directed in the operating conditions in the Purity.

Purity Nitrogen—Transfer 1.0 mL of Oxygen directly from cylinder with a pressure-reducing valve to gas-measuring tube or syringe for gas chromatography, using a polyvinyl chloride induction tube. Perform the test with this gas as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak area A₇ of nitrogen. Introduce 0.50 mL of nitrogen into the gas mixer, draw carrier gas into the mixer to make exactly 100 mL, allow to mix thoroughly and use this gas as the standard mixed gas. Perform the test in the same manner with 1.0 mL of this mixture as directed above, and determine the peak area A₅ of nitrogen: A₇ is not larger than A₅.

Operating conditions—

Detector: A thermal-conductivity detector.

Column: A column 3 mm in inside diameter and 3 m in length, packed with zeolite for gas chromatography 250- to
355-μm in particle diameter (a porosity of 0.5 nm).

Column temperature: A constant temperature of about 50°C.

Carrier gas: Hydrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of nitrogen is about 5 minutes.

**System suitability**—

System performance: Introduce 0.5 mL of nitrogen into a gas mixer, add Oxygen to make 100 mL, and mix thoroughly. When the test is run with 1.0 mL of the mixture under the above operating conditions, oxygen and nitrogen are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 5 times with 1.0 mL of the standard mixed gas under the above operating conditions, the relative standard deviation of the peak area of nitrogen is not more than 2.0%.

**Assay**

(i) Apparatus—The apparatus is shown diagrammatically in the accompanying figure. A is a 100-mL gas buret having a two-way stopcock a, b – c, d – e, and e – f are graduated in 0.1 mL, and c – d is graduated in 2 mL. A is properly connected with a leveling tube B by a thick rubber tube. Fill ammonium chloride-ammonia TS up to the middle of A and B. Place in the absorption ball g of the gas pipette C a coil of copper wire, not more than 2 mm in diameter, which extends to the uppermost portion of the bulb, add 125 mL of ammonium chloride-ammonia TS, and stopper with a rubber stopper i. Connect C with A using the thick rubber tube.

(ii) Procedure—Open a, set B downward and draw the liquid in g to the stopcock opening a. Then close a. Open a to the intake tube h, and fill A and h with ammonium chloride-ammonia TS by lifting B. Close a, connect h with a container of Oxygen, open a, set B downward and measure accurately 100 mL of Oxygen. Open a toward C, and transfer the Oxygen to g by lifting B. Close a, and rock C gently for 5 minutes. Open a, draw the residual gas back into A by setting B downward, and measure the volume of the residual gas. Repeat the procedure until the volume of residual gas is constant, and designate this as \( V \) (mL). With fresh ammonium chloride-ammonia TS in C, repeat the procedure at least four times, and measure the volume of residual gas. Calculate the volume of Oxygen and \( V \) in the following formula on the basis of the gas volume at 20°C and at 101.3 kPa.

\[
\text{Volume (mL) of oxygen (O}_2\) = \text{volume of Oxygen (mL) - } V \text{ (mL)}
\]

**Containers and storage**

Containers—Cylinders.

Storage—Not exceeding 40°C.

**Oxymetholone**

オキシメトロン

\( \text{C}_21\text{H}_32\text{O}_3 \): 332.48
17β-Hydroxy-2-hydroxymethylene-17α-methyl-5α-androstan-3-one

[434-07-1]

Oxymetholone, when dried, contains not less than 97.0% and not more than 103.0% of \( \text{C}_21\text{H}_32\text{O}_3 \).

**Description** Oxymetholone occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in 1,4-dioxane, sparingly soluble in methanol, in ethanol (95) and in acetone, slightly soluble in diethyl ether, and practically insoluble in water.

It is gradually colored and decomposed by light.

**Identification**

1. Dissolve 2 mg of Oxymetholone in 1 mL of ethanol (95), and add 1 drop of iron (III) chloride TS: a purple color develops.

2. Dissolve 0.01 g of Oxymetholone in methanol to make 50 mL. To 5 mL of the solution add 5 mL of sodium hydroxide-methanol TS and methanol to make 50 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry [2.24], and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

3. Determine the infrared absorption spectrum of Oxymetholone as directed in the potassium bromide disk method under Infrared Spectrophotometry [2.25], and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** [2.49] \([\alpha]_{D}^{20} : +34 – +38°\) (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

**Melting point** [2.60] 175 – 182°C.

**Purity**

1. Clarity and color of solution—Dissolve 0.5 g of Oxymetholone in 25 mL of 1,4-dioxane: the solution is clear, and shows a colorless to pale yellow color.
(2) Related substances—Dissolve 50 mg of Oxymetholone in 5 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.03). Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and air-dry the spot. Develop immediately the plate with a mixture of toluene and ethanol (99:5) (49:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, and heat at 100°C for 3 to 5 minutes: any spot other than the principal spot and starting point obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying (2.41) Not more than 1.0% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition (2.44) Not more than 0.1% (0.5 g).

Assay Weigh accurately about 40 mg of Oxymetholone, previously dried, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, and add methanol to make exactly 50 mL. To exactly measured 5 mL of this solution add 5 mL of sodium hydroxide-methanol TS and methanol to make exactly 50 mL. Determine the absorbance at 245 nm with an ultraviolet-visible spectrophotometer (2.24), using a solution, prepared by adding methanol to 5 mL of sodium hydroxide-methanol TS to make 50 mL, as the blank.

Amount (mg) of C_{21}H_{35}O_{3} = A/541 × 50,000

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

**Oxytetracycline Hydrochloride**

オキシテトラサイクリン塩酸塩

![Chemical Structure](image)

Oxytetracycline Hydrochloride is the hydrochloride of a tetracycline substance having antibacterial activity produced by the growth of *Streptomyces rimosus*.

It contains not less than 880 μg (potency) and not more than 945 μg (potency) per mg, calculated on the dried basis. The potency of Oxytetracycline Hydrochloride is expressed as mass (potency) of oxytetracycline (C_{22}H_{24}N_{2}O_{9}: 460.43).

**Description** Oxytetracycline Hydrochloride occurs as yellow, crystals or crystalline powder.

It is freely soluble in water, and slightly soluble in ethanol (99.5).

**Identification** (1) Determine the absorption spectrum of a solution of Oxytetracycline Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Oxytetracycline Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 20 mg of Oxytetracycline Hydrochloride in 3 mL of water, and add 1 drop of silver nitrate TS: a white turbidity is produced.

**Optical rotation** (2.44) [α]_D^20: −188 – −200° (0.25 g calculated on the dried basis, 0.1 mol/L hydrochloric acid, 25 mL, 100 mm).

**Purity** (1) Heavy metals (1.07)—Proceed with 0.5 g of Oxytetracycline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 50 ppm).

(2) Related substances—Dissolve 20 mg of Oxytetracycline Hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, dissolve 20 mg of 4-epioxytetracycline in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as 4-epioxytetracycline stock solution. Separately, dissolve 20 mg of tetracycline hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as tetracycline hydrochloride stock solution. Separately, dissolve 8 mg of β-apooxytetracycline in 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as β-apooxytetracycline stock solution. Pipet 1 mL of 4-epioxytetracycline stock solution, 4 mL of tetracycline hydrochloride stock solution and 40 mL of β-apooxytetracycline stock solution, add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.01) according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of 4-epioxytetracycline and tetracycline obtained from the sample solution are not larger than each of the peak area from the standard solution, and the total area of the peaks, α-apooxytetracycline having the relative retention time of about 2.1 with respect to oxytetracycline, β-apooxytetracycline and the peaks, which appear between α-apooxytetracycline and β-apooxytetracycline, is not larger than the peak area of β-apooxytetracycline from the standard solution. The peak area of 2-acetyl-2-decarboxamide oxytetracycline, which appears after the principal peak, obtained from the sample solution is not larger than 4 times the peak area of 4-epioxytetracycline from the standard solution.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene
Oxytetracycline Hydrochloride / Official Monographs

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with strongly acidic ion exchange resin for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase A: Dissolve 3.402 g of potassium dihydrogen phosphate and 9.306 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 700 mL of water, add 300 mg of disodium dihydrogen ethylenediamine tetraacetate dihydrate to make 1000 mL.

Mobile phase B: Mix 60 mL of 0.33 mol/L potassium dihydrogen phosphate TS, 50 mL of a solution of tetrabutylammonium hydrogensulfate (1 in 100), 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 2500) and 200 mL of water, and adjust the pH to 7.5 with 2 mol/L sodium hydroxide TS. To this solution add 30 g of t-butanol and water to make 1000 mL.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–20</td>
<td>70 → 10</td>
<td>30 → 90</td>
</tr>
<tr>
<td>20–35</td>
<td>10 → 20</td>
<td>90 → 80</td>
</tr>
</tbody>
</table>

Flow rate: 1.0 mL/min.

Time span of measurement: About 3.5 times as long as the retention time of oxytetracycline beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of 4-epoxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. Pipet 4 mL of this solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Confirm that the peak area of 4-epoxytetracycline obtained from 20 μL of this solution is equivalent to 14 to 26% of that from 20 μL of the standard solution.

System performance: Dissolve 8 mg of α-apoepoxytetracycline in 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid TS to make 100 mL, and use this solution as α-apoepoxytetracycline stock solution. Mix 3 mL of the sample solution, 2 mL of 4-epoxytetracycline stock solution, 6 mL of tetracycline hydrochloride stock solution, 6 mL of β-apoapoytetracycline stock solution and 6 mL of α-apoapoytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, 4-epoxytetracycline, oxytetracycline, tetracycline, α-apoepoxytetracycline and β-apoapoytetracycline are eluted in this order with the resolutions between the peaks, 4-epoxytetracycline and oxytetracycline, oxytetracycline and tetracycline, and α-apoepoxytetracycline and β-apoapoytetracycline being not less than 4, not less than 5 and not less than 4, respectively, and the symmetry factor of the peak of oxytetracycline is not more than 1.3.

System repeatability: Pipet 1 mL of 4-epoxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. When the test is repeated 6 times with 20 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of 4-epoxytetracycline is not more than 2.0%.

Loss on drying <2.4% Not more than 2.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.4% Not more than 0.5% (1 g).

Assay Weigh accurately an amount of Oxytetracycline Hydrochloride and Oxytetracycline Hydrochloride RS, equivalent to about 50 mg (potency), and dissolve each in diluted hydrochloric acid (1 in 100) to make exactly 50 mL. Pipet 5 mL of each of these solutions, add diluted methanol (3 in 20) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.91> according to the following conditions, and determine the peak areas, A T and A S, of oxytetracycline.

Amount [μg (potency)] of oxytetracycline (C22H24N2O9)

\[
M_S = \frac{M_T}{A_T/A_S} \times 1000
\]

M S: Amount [mg (potency)] of Oxytetracycline Hydrochloride RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with strongly acidic ion exchange resin for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 3.402 g of potassium dihydrogen phosphate and 9.306 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 700 mL of water, add 300 mL of methanol, and adjust the pH to 4.5 with dilute hydrochloric acid.

Flow rate: Adjust the flow rate so that the retention time of oxytetracycline is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the theoretical plates and the symmetrical coefficient of the peak of oxytetracycline are not less than 1000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytetracycline is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.
Oxytocin
オキシトシン

C₄₃H₆₆N₁₂O₁₂S₂: 1007.19
[50-56-6]

Oxytocin is a synthetic peptide having the property of causing the contraction of uterine smooth muscle.

It contains not less than 540 oxytocin Units and not more than 600 oxytocin Units per mg, calculated on the dehydrated and de-acetic acid basis.

**Description**

Oxytocin occurs as a white powder. It is very soluble in water, and freely soluble in ethanol (99.5). It dissolves in hydrochloric acid TS. The pH of a solution prepared by dissolving 0.10 g of Oxytocin in 10 mL of freshly boiled and cooled water is between 4.0 and 6.0. It is hygroscopic.

**Identification**

Determine the absorption spectrum of a solution of Oxytocin (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Constituent amino acids**

Put about 1 mg of Oxytocin in a test tube for hydrolysis, add 6 mol/L hydrochloric acid TS to dissolve, replace the air in the tube with Nitrogen, remove the air in the test tube for hydrolysis, add 6 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 27 mg of L-aspartic acid, about 24 mg of L-threonine, about 21 mg of L-serine, about 29 mg of L-glutamic acid, about 23 mg of L-proline, about 15 mg of glycine, about 18 mg of L-alanine, about 23 mg of L-valine, about 48 mg of L-cystine, about 30 mg of methionine, about 26 mg of L-isoleucine, about 26 mg of L-leucine, about 36 mg of L-tyrosine, about 33 mg of phenylalanine, about 37 mg of L-lysine hydrochloride, about 42 mg of L-histidine hydrochloride monohydrate and about 42 mg of L-arginine hydrochloride, dissolve them in 10 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the respective molar ratios with respect to leucine: 0.95 – 1.05 for aspartic acid, 0.95 – 1.05 for glutamic acid, 0.95 – 1.05 for proline, 0.95 – 1.05 for glycine, 0.80 – 1.10 for isoleucine, 0.80 – 1.05 for tyrosine and 0.80 – 1.05 for cystine, and not more than 0.01 each for others.

**Operating conditions**

Detector: A visible spectrophotometer (wavelength: 440 nm and 570 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (sodium type) composed with a sulfonated polystyrene copolymer (3 µm in particle diameter).

Column temperature: A constant temperature of about 57°C.

Chemical reaction bath temperature: A constant temperature of about 130°C.

Color developing time: About 1 minute.

Mobile phase: Prepare mobile phases A, B and C according to the following table.

<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid monohydrate</td>
<td>19.80 g</td>
<td>22.00 g</td>
<td>6.10 g</td>
</tr>
<tr>
<td>Trisodium citrate dihydrate</td>
<td>6.19 g</td>
<td>7.74 g</td>
<td>26.67 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.66 g</td>
<td>7.07 g</td>
<td>54.35 g</td>
</tr>
<tr>
<td>Ethanol (99.5)</td>
<td>260.0 mL</td>
<td>20.0 mL</td>
<td>—</td>
</tr>
<tr>
<td>Benyl alcohol</td>
<td>—</td>
<td>—</td>
<td>5.0 mL</td>
</tr>
<tr>
<td>Thiodiglycol</td>
<td>5.0 mL</td>
<td>5.0 mL</td>
<td>—</td>
</tr>
<tr>
<td>Lauromacrogol solution (1 in 4)</td>
<td>4.0 mL</td>
<td>4.0 mL</td>
<td>4.0 mL</td>
</tr>
<tr>
<td>Caprylic acid</td>
<td>0.1 mL</td>
<td>a sufficient amount</td>
<td>a sufficient amount</td>
</tr>
<tr>
<td>Water</td>
<td>a sufficient amount</td>
<td>a sufficient amount</td>
<td>a sufficient amount</td>
</tr>
</tbody>
</table>

**Flowing of the mobile phase**: Control the gradient by mixing the mobile phases A, B and C as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
<th>Mobile phase C (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 9</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9 – 25</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>25 – 61</td>
<td>0</td>
<td>100 → 0</td>
<td>0 → 100</td>
</tr>
<tr>
<td>61 – 80</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Reaction reagent: Mix 407 g of lithium acetate dihydrate, 245 mL of acetic acid (100) and 801 mL of 1-methoxy-2-propanol, add water to make 2000 mL, stir for more than 10 minutes while passing Nitrogen, and use this solution as Solution A. Separately, to 1957 mL of 1-methoxy-2-propanol, add 77 g of ninhydrin and 0.134 g of sodium borohydride, stir for more than 10 minutes while passing Nitrogen, and use this solution as Solution B. Mix Solution A and Solution B before use.

Flow rate of mobile phase: About 0.26 mL per minute.

Flow rate of reaction reagent: About 0.3 mL per minute.

**System suitability**

System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine are eluted in this order with the resolutions between the peaks.
peaks of threonine and serine, glycine and alanine, and isoleucine and leucine being not less than 1.5, 1.4 and 1.2, respectively.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak area of aspartic acid, proline, valine and arginine are not more than 2.0%, respectively.

**Purity (1)** Acetic acid—Weigh accurately about 15 mg of Oxytocin, dissolve in the internal standard solution to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 1 g of acetic acid (100), add the internal standard solution to make exactly 100 mL. Pipet 2 mL of this solution, add the internal standard solution to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \(Q_2\) and \(Q_3\), of the peak area of acetic acid to that of the internal standard: the amount of acetic acid is not less than 6.0% and not more than 10.0%.

\[
\text{Amount (\%)} = \frac{M_s}{M_T} \times \frac{Q_T}{Q_s} \times \frac{1}{10}
\]

**Internal standard solution**—A solution of propionic acid in the mobile phase (1:10,000).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: To 0.7 mL of phosphoric acid add 900 mL of water, adjust the pH to 3.0 with 8 mol/L sodium hydroxide TS, and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of acetic acid is about 3 minutes.

**System suitability**—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, acetic acid and propionic acid are eluted in this order with the resolution between these peaks being not less than 14.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of acetic acid to that of the internal standard is not more than 2.0%.

(2) Related substances—Dissolve 25 mg of Oxytocin in 100 mL of the mobile phase A, and use this solution as the sample solution. Perform the test with 50 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of each peak other than Oxytocin is not more than 1.5%, and the total of them is not more than 5.0%.

**Operating conditions**—
Detector, column, column temperature, mobile phase, flowing of mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

**Time span of measurement**—About 2.5 times as long as the retention time of oxytocin.

**System suitability**—
Test for required detectability: Measure exactly 1 mL of the sample solution, add the mobile phase A to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase A to make exactly 10 mL. Confirm that the peak area of oxytocin obtained from 50 μL of this solution is equivalent to 5 to 15% of that from 50 μL of the solution for system suitability test.

System performance: Dissolve an adequate amount of oxytocin and vasopressin in the mobile phase A, so that each mL contains about 0.1 mg each of them. When the procedure is run with 50 μL of this solution under the above operating conditions, vasopressin and oxytocin are eluted in this order with the resolution between these peaks being not less than 14, and the symmetry factor of the peak of oxytocin is not more than 1.5.

System repeatability: When the test is repeated 6 times with 50 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of oxytocin is not more than 2.0%.

**Water** <2.48> Not more than 5.0% (50 mg, coulometric titration).

**Assay**—Weigh accurately an amount of Oxytocin, equivalent to about 13,000 Units, dissolve in the mobile phase A to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 1 bottle of the Oxytocin RS in the mobile phase A to make a known concentration solution containing each mL contains about 130 Units, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, \(A_1\) and \(A_3\), of oxytocin.

Units per mg of Oxytocin, calculated on the dehydrated and de-acetic acid basis

\[
M_s = \frac{M_T}{M_1} \times \frac{A_1}{A_3} \times 100
\]

\(M_s\): Units per mL of the standard solution
\(M_1\): Amount (mg) of sample, calculated on the dehydrated and de-acetic acid basis

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 220 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase A: Dissolve 15.6 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water.
Mobile phase B: A mixture of water and acetonitrile (1:1). Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.
Flow rate: About 1.0 mL per minute.

System suitability—

System performance: Dissolve 2 mg each of oxytocin and vasopressin in 20 mL of the mobile phase A. When the procedure is run with 25 μL of this solution under the above operating conditions, vasopressin and oxytocin are eluted in this order with the resolution between these peaks being not less than 14, and the symmetry factor of the peak of oxytocin is not more than 1.5.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytocin is not more than 1.0%.

Containers and storage—Containers—Tight containers.

Storage—At 2 to 8°C.

---

**Oxytocin Injection**

オキシトシン注射液

Oxytocin Injection is an aqueous solution for injection.

It contains not less than 90.0% and not more than 110.0% of the labeled oxytocin Units.

**Method of preparation** Prepare as directed under Injections, with Oxytocin.

**Description** Oxytocin Injection is a colorless, clear liquid.

pH <2.5 – 2.5 – 4.5

**Bacterial endotoxins** <4.01> Less than 10 EU/oxytocin Unit.

**Extractable volume** <6.05> It meets the requirement.

**Foreign insoluble matter** <6.06> Perform the test according to the Method 1: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Measure exactly a portion of Oxytocin Injection according to the labeled Units, dilute with the diluent so that each mL contains about 1 Unit, and use this solution as the sample solution. Separately, dissolve 1 bottle of Oxytocin RS in the mobile phase A to make exactly 20 mL. Pipet a suitable volume of this solution, dilute with the diluent to make a known concentration solution so that each mL contains about 1 Unit, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 30</td>
<td>70 → 40</td>
<td>30 → 60</td>
</tr>
<tr>
<td>30 – 30.1</td>
<td>40 → 70</td>
<td>60 → 30</td>
</tr>
<tr>
<td>30.1 – 45</td>
<td>70</td>
<td>30</td>
</tr>
</tbody>
</table>

Flow rate: About 1.0 mL per minute.

**System suitability**—

System performance: Dissolve 2 mg each of oxytocin and vasopressin in 100 mL of the mobile phase A. When the procedure is run with 100 μL of this solution under the above operating conditions, vasopressin and oxytocin are eluted in this order with the resolution between these peaks being not less than 14, and the symmetry factor of the peak of oxytocin is not more than 1.5.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytocin is not more than 2.0%.

**Containers and storage** Containers—Hermetic containers.

Storage—In a cold place, and avoid freezing.

---

**Official Monographs / Oxytocin Injection** 1205

determine the peak areas, \( A_T \) and \( A_S \), of oxytocin.

Units per mL of Oxytocin Injection

\[
M_S = M_S = \frac{A_T}{A_S} \times \frac{b}{a} 
\]

\( M_S \): Units per mL of the standard solution

\( a \): Volume (mL) of sample

\( b \): Total volume of the sample solution prepared by diluting with the diluent

**Diluent** Dissolve 5 g of chlorobutanol, 1.1 g of sodium acetate trihydrate, 5 g of acetic acid (100) and 6 mL of ethanol (99.5) in water to make 1000 mL.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 15.6 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water.

Mobile phase B: A mixture of water and acetonitrile (1:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 30</td>
<td>70 → 40</td>
<td>30 → 60</td>
</tr>
<tr>
<td>30 – 30.1</td>
<td>40 → 70</td>
<td>60 → 30</td>
</tr>
<tr>
<td>30.1 – 45</td>
<td>70</td>
<td>30</td>
</tr>
</tbody>
</table>
Ozagrel Sodium

オザグレルナトリウム

\[ \text{C}_{13}\text{H}_{11}\text{N}_{2}\text{NaO}_{2} : 250.23 \]

Monosodium (2E)-3-[4-(1H-imidazol-1-ylmethyl)phenyl]prop-2-enoate

[189224-26-8]

Ozagrel Sodium, when dried, contains not less than 98.0% and not more than 102.0% of \( \text{C}_{13}\text{H}_{11}\text{N}_{2}\text{NaO}_{2} \).

**Description**

Ozagrel Sodium occurs as white crystals or crystalline powder.

It is freely soluble in water, soluble in methanol, and practically insoluble in ethanol (99.5).

**Identification** (1) Determine the absorption spectrum of a solution of Ozagrel Sodium (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\rangle\), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ozagrel Sodium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ozagrel Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\rangle\), and compare the spectrum with the Reference Spectrum or the spectrum of Ozagrel Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ozagrel Sodium (1 in 20) responds to the Qualitative Tests \(<1.09\rangle\) for sodium salt.

**pH** \(<2.54\rangle\)

The pH of a solution prepared by dissolving 0.5 g of Ozagrel Sodium in 10 mL of water is between 9.5 and 10.5.

**Purity** (1) Clarity and color of solution—Dissolve 0.5 g of Ozagrel Sodium in 10 mL of water: the solution is clear and colorless.

(2) Chloride \(<1.03\rangle\)—Dissolve 2.0 g of Ozagrel Sodium in 30 mL of water, add 1 mL of acetic acid (100) and water to make 50 mL, shake, and allow to stand for 30 minutes. Filter the solution, discard the first 5 mL of the filtrate, and to 25 mL of the subsequent filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution as follows: To 0.35 mL of 0.01 mol/L hydrochloric acid VS add 0.5 mL of acetic acid (100), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.012%).

(3) Heavy metals \(<1.07\rangle\)—Proceed with 2.0 g of Ozagrel Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 50 mg of Ozagrel Sodium in 100 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 5 \( \mu \)L of the sample solution as directed under Liquid Chromatography \(<2.03\rangle\) according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: each of the amount other than ozagrel is not more than 0.2%, and the total amount other than ozagrel is not more than 0.5%.

**Operating conditions**—

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Time span of measurement: About 2 times as long as the retention time of ozagrel, beginning after the solvent peak.

**System suitability**—

Test for required detectability: Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 200 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of ozagrel obtained from 5 \( \mu \)L of this solution is equivalent to 15 to 25% of that from 5 \( \mu \)L of the solution for system suitability test.

System performance: When the procedure is run with 5 \( \mu \)L of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ozagrel are not less than 6000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 5 \( \mu \)L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of ozagrel is not more than 2.0%.

**Loss on drying** \(<2.41\rangle\)

Not more than 0.5% (1 g, 105°C, 4 hours).

**Assay**

Weigh accurately about 25 mg each of Ozagrel Sodium and Ozagrel Sodium RS, both previously dried, and dissolve each in methanol to make exactly 25 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 1 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.04\rangle\) according to the following conditions, and calculate the ratios, \( Q_t \) and \( Q_S \), of the peak area of ozagrel to that of the internal standard.

\[
\text{Amount (mg)} = M_S \times \frac{Q_t}{Q_S}
\]

\( M_S \): Amount (mg) of Ozagrel Sodium RS

**Internal standard solution**—A solution of benzoic acid in methanol (1 in 100).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of ammonium acetate (3 in 1000) and methanol (4:1).

Flow rate: Adjust the flow rate so that the retention time of ozagrel is about 10 minutes.

**System suitability**—

System performance: When the procedure is run with 1 \( \mu \)L of the standard solution under the above operating condi-
Ozagrel Sodium for Injection

注射用オザグレルナトリウム

Ozagrel Sodium for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of ozagrel sodium ($C_{13}H_{11}N_{2}NaO_{2}$: 250.23).

Method of preparation: Prepare as directed under Injections, with Ozagrel Sodium.

Description: Ozagrel Sodium for Injection occurs as white masses or powder.

Identification: Dissolve an amount of Ozagrel Sodium for Injection, equivalent to 40 mg of Ozagrel Sodium according to the labeled amount, in water to make 40 mL. To 1 mL of this solution add water to make 200 mL, and determine the absorption spectrum of this solution as directed under Ultra-violet-visible Spectrophotometry: it exhibits a maximum between 269 nm and 273 nm.

pH: Being specified separately.

Purity: Related substances—Dissolve an amount of Ozagrel Sodium for Injection, equivalent to 0.20 g of Ozagrel Sodium according to the labeled amount, in the mobile phase to make 100 mL. To 5 mL of this solution add the mobile phase to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Purity (4) under Ozagrel Sodium.

Bacterial endotoxins: Less than 3.7 EU/mg.

Uniformity of dosage units: It meets the requirement of the Mass variation test.

Foreign insoluble matter: Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter: It meets the requirement.

Sterility: Perform the test according to the Membrane filtration method: it meets the requirement.

Assay: Dissolve an amount of Ozagrel Sodium for Injection, equivalent to about 0.4 g of ozagrel sodium ($C_{13}H_{11}N_{2}NaO_{2}$), in water to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and 5 mL of water, mix, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Ozagrel Sodium RS, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Then, proceed as directed in the Assay under Ozagrel Sodium.

Amount (mg) of ozagrel sodium ($C_{13}H_{11}N_{2}NaO_{2}$) = $M_S \times Q_T/Q_s \times 16$

$M_S$: Amount (mg) of Ozagrel Sodium RS

Internal standard solution—A solution of benzoic acid in methanol (1 in 100).

Containers and storage: Containers—Tight containers.

Storage: Light-resistant.

Pancreatin

パンクレアチン

Pancreatin is a substance containing enzymes prepared from the pancreas of edible animals, mostly the hog, and has amylolytic, proteolytic and lipolytic activities.

It contains not less than 2800 starch saccharifying activity units, not less than 28,000 proteolytic activity units, and not less than 960 lipolytic activity units per g.

It is usually diluted with suitable excipients.

Description: Pancreatin occurs as a white to light yellow powder. It has a characteristic odor.

Purity (1): Rancidity—Pancreatin has no unpleasant or rancid odor and is tasteless.

(2) Fat—Add 20 mL of diethyl ether to 1.0 g of Pancreatin, extract with occasional shaking for 30 minutes, and filter. Wash the residue with 10 mL of diethyl ether, combine the washing with the filtrate, evaporate the diethyl ether, and dry the residue at 105°C for 2 hours: the mass of the residue does not exceed 20 mg.

Loss on drying: Not more than 4.0% (1 g, in vacuum, phosphorus (V) oxide, 24 hours).

Residue on ignition: Not more than 5% (1 g).

Assay (1): Starch digestive activity

(i) Substrate solution—Use potato starch TS for amylolytic activity test, prepared by adding 10 mL of phosphate buffer solution for pancreatin instead of 10 mL of 1 mol/L acetic acid-sodium acetate buffer solution, pH 5.0.

(ii) Sample solution—Weigh accurately about 0.1 g of Pancreatin, add a suitable amount of ice-cold water, stir, and add ice-cold water to make exactly 100 mL. Pipet 10 mL of this solution, and add ice-cold water to make exactly 100 mL.


(2) Protein digestive activity

(i) Substrate solution—Use the substrate solution 2 described in (2) Assay for protein digestive activity under Digestion Test after adjusting the pH to 8.5.

(ii) Sample solution—Weigh accurately about 0.1 g of Pancreatin, add a suitable amount of ice-cold water, stir,
Pancuronium Bromide

パニペネム

C₃₅H₆₀Br₂N₂O₄: 732.67
1,1′-(3α,17β-Diacetoxy-5α-androstan-2β,16β-diyl)bis(1-methylpiperidinium) dibromide

[15500-66-0]

Pancuronium Bromide occurs as a white crystalline powder. It is very soluble in water, and freely soluble in ethanol (95) and in acetic anhydride. It is hygroscopic.

Identification (1) Determine the infrared absorption spectrum of Pancuronium Bromide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Pancuronium Bromide (1 in 100) responds to the Qualitative Tests <1.09> (1) for bromide.

Optical rotation <2.49> [α]D: +38° to +42° (0.75 g calculated on the dehydrated basis, water, 25 mL, 100 mm).

pH <2.50> The pH of a solution of Pancuronium Bromide (1 in 100) is between 4.5 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Pancuronium Bromide in 10 mL of water: the solution is clear and colorless.

(2) Related substances—Dissolve 50 mg of Pancuronium Bromide in 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution (1). Separately, weigh exactly 5 mg of pancuronium bromide for thin-layer chromatography, add ethanol (95) to make exactly 25 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 2 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, acetonitrile and a solution of sodium iodide (1 in 5) (17:2:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of sodium nitrite in methanol (1 in 100) on the plate, allow to stand for 2 minutes, and spray evenly potassium bromide TS on the plate: a spot from the sample solution, corresponding to that from the standard solution (2), has no more color than that from the standard solution (2), and the spots other than the principal spot and the above mentioned spot from the sample solution have no more color than the spot from the standard solution (1).

Water <2.48> Not more than 8.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Pancuronium Bromide, dissolve in 50 mL of acetic anhydride by warming, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 36.63 mg of C₃₅H₆₀Br₂N₂O₄

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Panipenem

パニペネム

C₁₅H₂₁N₉O₅S: 339.41
(SR,6S)-6-[(1R)-1-Hydroxyethyl]3-[(3S)-1-(1-iminoethyl)pyrrolidin-3-ylsulfanyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid [87726-17-8]

Panipenem contains not less than 900 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the anhydrous basis and corrected on the amount of the residual solvent. The potency of Panipenem is expressed as mass (potency) of panipenem (C₁₅H₂₁N₉O₅S).

Description Panipenem occurs as a white to light yellow, crystalline powder or mass.
It is very soluble in water, freely soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether. It is hygroscopic.

It deliquesces in the presence of moisture.

**Identification (1)** Dissolve 0.02 g of Panipenem in 2 mL of water, add 1 mL of hydroxyammonium chloride-ethanol TS, allow to stand for 3 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown color develops.

(2) Determine the absorption spectrum of a solution of Panipenem in 0.02 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution, pH 7.0 (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 296 nm and 300 nm.

(3) Determine the infrared absorption spectrum of Panipenem as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1760 cm⁻¹, 1676 cm⁻¹, 1632 cm⁻¹, 1588 cm⁻¹, 1384 cm⁻¹ and 1249 cm⁻¹.

**Residual solvents** <2.41>—Follow the test for Residual Solvents in separated parts of 1-propanol is about 6 minutes.

| Operating conditions | Detector: Hydrogen flame-ionization detector. | Flow rate: Adjust the flow rate so that the retention time of 1-propanol is about 6 minutes. | System suitability— | System performance: When the procedure is run with 1 mL of the gas of the standard solution (2) under the above operating conditions, ethanol, acetone and the internal standard are eluted in this order with the resolution between ethanol and acetone being not less than 4. | System repeatability: When the test is repeated 6 times with 1 mL of the gas of the standard solution (2) under the above operating conditions, the relative standard deviation of the ratios of the peak area of ethanol to that of the internal standard is not more than 5.0%. |

**Purity (1)** Clarity and color of solution—Being specified separately.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Panipenem according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Residual solvents <2.46>—Weigh accurately about 0.2 g of Panipenem, transfer to a 20-mL narrow-mouthed cylindrical glass bottle, add exactly 2 mL of the internal standard solution and 2 mL of water to dissolve, seal tightly a rubber stopper with aluminum cap, and use this solution as the sample solution. Separately, pipet 15 mL of ethanol (99.5) and 3 mL of acetone, add water to make exactly 200 mL, Pipet 1 mL and 2 mL of this solution, and add water to them to make exactly 20 mL. Transfer exactly 2 mL each of these solutions to a 20-mL narrow-mouthed cylindrical glass bottle, add exactly 2 mL of the internal standard solution, seal tightly a rubber stopper with aluminum cap, and use these solutions as the standard solution (1) and the standard solution (2). Shake gently the sample solution and the standard solutions (1) and (2) in a water bath at a constant room temperature, and allow to stand for 30 minutes. Perform the test with 1 mL of the gas in each container as directed under Gas Chromatography <2.02> according to the following conditions. Calculate the ratios, Q₁, Q₂, and Q₃, of the peak area of ethanol and acetone to that of the internal standard from the sample solution, the ratios, Q₁, Q₂, and Q₃₁, of the peak area of ethanol and acetone to that of the internal standard from the standard solution (1), and the ratios, Q₂ and Q₃₂, of the peak area of ethanol and acetone to that of the internal standard from the standard solution (2). Calculate the amount of the ethanol and acetone by the following formula: ethanol is not more than 5.0% and acetone is not more than 1.0%.  

\[
\text{Amount (\%)} = \frac{M_2}{M_1} \times \frac{(Q₁ + Q₂)}{2(Q₂ - Q₃)} \\
\text{Amount (\%)} = \frac{1/100 \times 100}{1/100 \times 100} \\
\]

**M₁**: Amount (g) of Panipenem  

**M₂**: Amount (g) of water  

**Internal standard solution**—A solution of 1-propanol (1 in 400).
Pantethine

Panethine

Pantethine is an aqueous solution containing 80% of pantethine. Pantethine contains not less than 98.0% of pantethine (C₂₂H₄₂N₄O₈S₂), calculated on the anhydrous basis.

Description

Pantethine is a clear, colorless to pale yellow viscous liquid.

It is miscible with water, with methanol and with ethanol (95). It is decomposed by light.

Identification

1. To 0.7 g of Pantethine add 5 mL of sodium hydroxide TS, shake, and add 1 to 2 drops of copper (II) sulfate TS: a blue-purple color develops.

2. To 0.7 g of Pantethine add 3 mL of water, shake, add 0.1 g of zinc powder and 2 mL of acetic acid (100), and boil for 2 to 3 minutes. After cooling, add 1 to 2 drops of sodium pentacyanonitrosylferrate (III) TS: a red-purple color develops.

3. To 1.0 g of Pantethine add 500 mL of water, and shake. To 5 mL of this solution add 3 mL of 1 mol/L hydrochloric acid TS, and heat on a water bath for 30 minutes. After cooling, add 7 mL of a solution of hydroxylammonium chloride in sodium hydroxide TS (3 in 140), and allow to stand for 5 minutes. Add 3 drops of 2,4-dinitrophenol TS, and add 1 mol/L hydrochloric acid TS dropwise until the solution has no color, and then add 1 mL of iron (III) chloride TS: a red-purple color develops.

Optical rotation

[α]D: +15.0° to +18.0° (1 g calculated on the anhydrous basis, water, 25 mL, 100 mm).
Purity (1) Heavy metals \(<1.07\) — Proceed with 2.0 g of Pantethine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic \(<1.17\) — Prepare the test solution with 2.0 g of Pantethine according to Method 3, and perform the test (not more than one ppm).

(3) Related substances — Dissolve 0.6 g of Pantethine in 10 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.07\>.

(4) Mercapto compounds — To 1.5 g of Pantethine add 20 mL of water, shake, add 1 drop of ammonia TS and 1 to 2 drops of sodium pentacyanoferrate (III) TS: a red color is not developed.

Water \(<2.48\> 18 - 22\% \,(0.2\, g, \, volumetric titration, \, direct titration).

Residue on Ignition \(<2.44\> \, \text{Not more than 0.1\%} \,(2\, g).

Assay Weigh accurately about 0.3 g of Pantethine, add water to make exactly 20 mL. Transfer exactly 5 mL of this solution in an iodine bottle, and add exactly 25 mL of 0.05 mol/L bromine VS and 100 mL of water. Add 5 mL of diluted sulfuric acid (1 in 5) rapidly, stopper tightly immediately, and warm at 40 to 50°C for 15 minutes with occasional shaking. After cooling, carefully add 5 mL of a solution of potassium iodide (2 in 5), then immediately stopper tightly, shake, add 100 mL of water and titrate \(<2.50\> \, \text{the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS). Perform a blank determination.}

Each mL of 0.05 mol/L bromine VS = 5.547 mg of \(C_{22}H_{42}N_4O_8S_2\)

Containers and storage Containers — Tight containers.

Storage — Light-resistant, at a temperature not exceeding 10°C.

Papaverine Hydrochloride パパベリン塩酸塩

C\(_2\)H\(_3\)NO\(_3\)HCl: 375.85

6,7-Dimethoxy-1-(3,4-dimethoxybenzyl)isoquinoline monohydrochloride

\([61-25-6]\)

Papaverine Hydrochloride, when dried, contains not less than 98.5\% of \(C_{30}H_{33}NO_4\cdot HCl\).

Description Papaverine Hydrochloride occurs as white crystals or crystalline powder.

It is sparingly soluble in water and in acetic acid (100), slightly soluble in ethanol (95), and practically insoluble in acetanhydride and in diethyl ether.

The pH of a solution of Papaverine Hydrochloride (1 in 50) is between 3.0 and 4.0.

Identification (1) To 1 mg of Papaverine Hydrochloride add 1 drops of formaldehyde-sulfuric acid TS: a colorless to light yellow-green color is produced, and it gradually changes to deep red, then to brown.

(2) Dissolve 0.02 g of Papaverine Hydrochloride in 1 mL of water, and add 3 drops of sodium acetate TS: a white precipitate is produced.

(3) Dissolve 1 mg of Papaverine Hydrochloride in 3 mL of acetic anhydride and 5 drops of sulfuric acid, heat in a water bath for 1 minute, and examine under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence.

(4) Dissolve 0.1 g of Papaverine Hydrochloride in 10 mL of water, make alkaline with ammonia TS, and shake with 10 mL of diethyl ether. Draw off the diethyl ether layer, wash with 5 mL of water, and filter. Evaporate the filtrate on a water bath, and dry the residue at 105°C for 3 hours: the residue so obtained melts \(<2.60\> \, \text{between 145°C and 148°C}.

(5) Alkalify a solution of Papaverine Hydrochloride (1 in 50) with ammonia TS, and filter the precipitate. Acidify the filtrate with dilute nitric acid: the solution responds to Qualitative Tests \(<1.09\> (2) for chloride.

Purity (1) Clarity and color of solution — Dissolve 0.10 g of Papaverine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Morphine — Dissolve 10 mg of Papaverine Hydrochloride in 1 mL of water, add 5 mL of 1-nitroso-2-naphthol TS and 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. Add 1 mL of a solution of sodium nitrate (1 in 5000), and warm at 40°C for 5 minutes. After cooling, shake the mixture with 10 mL of chloroform, centrifuge, and separate the aqueous layer: the solution so obtained has no more color than a pale red color.
Papaverine Hydrochloride Injection

パパベリン塩酸塩注射液

Papaverine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of papaverine hydrochloride (C₂₀H₂₁NO₄.HCl: 375.85).

Method of preparation Prepare as directed under Injections, with Papaverine Hydrochloride.

Description Papaverine Hydrochloride Injection is a clear, colorless liquid.

pH: 3.0–5.0

Identification (1) To 1 mL of Papaverine Hydrochloride Injection add 3 drops of sodium acetate TS: a white precipitate is produced.

(2) Dilute a volume of Papaverine Hydrochloride Injection, equivalent to 0.1 g of Papaverine Hydrochloride according to the labeled amount, with water to 10 mL, render the solution alkaline with ammonia TS, and shake with 10 mL of diethyl ether. Draw off the diethyl ether layer, wash with 5 mL of water, and evaporate the filtrate on a water bath to dryness, and dry the residue at 105°C. The residue so obtained melts between 145°C and 148°C.

(3) Proceed with 1 mg of each of the residue obtained in (2) as directed in the Identification (1) and (3) under Papaverine Hydrochloride.

(4) Alkalinize 2 mL of Papaverine Hydrochloride Injection with ammonia TS, filter the precipitate off, and acidify the filtrate with dilute nitric acid: the solution responds to Qualitative Tests (1.09) (2) for chloride.

Bacterial endotoxins (4.01) Less than 6.0 EU/mg.

Extractable volume (6.05) It meets the requirement.

Foreign insoluble matter (6.06) Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter (6.07) It meets the requirement.

Sterility (4.06) Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Dilute an exactly measured volume of Papaverine Hydrochloride Injection, equivalent to about 0.2 g of papaverine hydrochloride (C₂₀H₂₁NO₄.HCl), with water to 10 mL, render the solution alkaline with ammonia TS, and extract with 20-mL, 15-mL, 10-mL and 10-mL portions of chloroform. Combine the extracts, wash with 10 mL of water, and re-extract the washings with two 5-mL portions of chloroform. Combine all the chloroform extracts, and distil the chloroform on a water bath. Dissolve the residue in 30 mL of acetic acid (100), and titrate (2.50) with 0.05 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS = 18.79 mg of C₂₀H₂₁NO₄.HCl

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Paraffin

パラフィン

Paraffin is a mixture of solid hydrocarbons obtained from petroleum.

Description Paraffin occurs as a colorless or white, more or less transparent, crystalline mass. It is odorless and tasteless.

It is sparingly soluble in diethyl ether and practically insoluble in water, in ethanol (95) and in ethanol (99.5).

Specific gravity d₂₀° : about 0.92 (proceed as directed in 4.2. in 4. Specific gravity under Fats and Fatty Oils Test (1.13)).

Identification (1) Heat Paraffin strongly in a porcelain dish, and ignite: it burns with a bright flame and the odor of paraffin vapor is perceptible.

(2) Heat 0.5 g of Paraffin with 0.5 g of sulfur with shaking carefully: the odor of hydrogen sulfide is perceptible.

Melting point (2.60) 50–75°C (Method 2).

Purity (1) Acidity or alkalinity—Boil 10.0 g of Paraffin with 10 mL of hot water and 1 drop of phenolphthalein TS in a water bath for 5 minutes, and shake vigorously: a red color is not produced. Add 0.20 mL of 0.02 mol/L sodium hydroxide VS to this solution, and shake: a red color is produced.

(2) Heavy metals (1.07)—Ignite 2.0 g of Paraffin in a crucible, first moderately until charred, then between 450°C and 550°C to ash. Cool, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. To the residue add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(3) Arsenic (1.11)—Prepare the test solution with 1.0 g
of Paraffin according to Method 3, and perform the test (not more than 2 ppm).

(4) Sulfur compounds—To 4.0 g of Paraffin add 2 mL of ethanol (99.5), further add 2 drops of a clear saturated solution of lead (II) oxide in a solution of sodium hydroxide (1 in 5), and heat for 10 minutes at 70°C with occasional shaking: no dark brown color develops in the aqueous layer. Repeat this procedure four times: the color of the sulfuric acid layer is not darker than that of the following control solution.

Control solution: Add 1.5 mL of Cobalt (II) Chloride CS, 0.5 mL of Copper (II) Sulfate CS and 5 mL of liquid paraffin to 3.0 mL of Iron (III) Chloride CS, and shake vigorously.

Containers and storage Containers—Well-closed containers.

Liquid Paraffin

Liquid Paraffin is a mixture of liquid hydrocarbons obtained from petroleum.

Tocopherols of a suitable form may by added at a concentration not exceeding 0.001% as a stabilizer.

Description Liquid Paraffin is a colorless, transparent, oily liquid, nearly free from fluorescence. It is odorless and tasteless.

It is freely soluble in diethyl ether, very slightly soluble in ethanol (99.5), and practically insoluble in water and in ethanol (95).

Boiling point: above 300°C.

Identification (1) Heat Liquid Paraffin strongly in a porcelain dish, and fire: it burns with a bright flame and the odor of paraffin vapor is perceptible.

(2) Heat 0.5 of Liquid Paraffin with 0.5 g of sulfur with shaking carefully: the odor of hydrogen sulfide is perceptible.

Specific gravity <2.56> \( \rho_{20}^\circ \): 0.860 - 0.890

Viscosity <2.55> Not less than 37 mm²/s (Method 1, 37.8°C).

Purity (1) Odor—Transfer a suitable amount of Liquid Paraffin to a small beaker, and heat on a water bath: a foreign odor is not perceptible.

(2) Acidity or alkalinity—Shake vigorously 10 mL of Liquid Paraffin with 10 mL of hot water and 1 drop of phenolphthalein TS: no red color develops. Shake this solution with 0.20 mL of 0.02 mol/L sodium hydroxide VS: a red color develops.

(3) Heavy metals <1.07>—Ignite 2.0 g of Liquid Paraffin in a crucible, first moderately until charred, then between 450°C and 550°C to ash. Cool, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. To the residue add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Liquid Paraffin, according to Method 3 except that after addition of 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), add 1.5 mL of hydrogen peroxide (30), fire to burn, and perform the test (not more than 2 ppm).

(5) Solid paraffin—Transfer 50 mL of Liquid Paraffin, previously dried at 105°C for 2 hours, to a Nessler tube, and cool in ice water for 4 hours: the turbidity produced, if any, is not deeper than that of the following control solution.

Control solution: To 1.5 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, add 1 mL of silver nitrate TS, and allow to stand for 5 minutes.

(6) Sulfur compounds—Prepare a saturated solution of lead (II) oxide in a solution of sodium hydroxide (1 in 5), and mix 2 drops of this clear solution with 4.0 mL of Liquid Paraffin and 2 mL of ethanol (99.5). Heat at 70°C for 10 minutes with frequent shaking, and cool: no dark brown color develops.

(7) Polycyclic aromatic hydrocarbons—Take 25 mL of Liquid Paraffin by a 25-mL measuring cylinder, transfer to a 100-mL separator, and wash out the cylinder with 25 mL of hexane for ultraviolet-visible spectrophotometry. Combine the washings with the liquid in the separator, and shake vigorously. Shake this solution vigorously for 2 minutes with 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and allow to stand for 15 minutes. Transfer the lower layer to a 50-mL separator, add 2 mL of hexane for ultraviolet-visible spectrophotometry, shake vigorously for 2 minutes, and allow to stand for 2 minutes. Transfer the lower layer to a 10-mL glass-stoppered centrifuge tube, and centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution thus obtained as the sample solution. Transfer 25 mL of hexane for ultraviolet-visible spectrophotometry to another 50-mL separator, shake vigorously for 2 minutes with 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and allow to stand for 2 minutes. Transfer the lower layer to a 10-mL glass-stoppered centrifuge tube, centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution thus obtained as a control solution. Immediately determine the absorbance of the sample solution using the control solution as the blank as directed under Ultraviolet-visible Spectrophotometry <2.24>: not more than 0.10 at the wavelength region between 260 nm and 350 nm.

(8) Readily carbonizable substances—Transfer 5 mL of Liquid Paraffin to a Nessler tube, and add 5 mL of sulfuric acid for readily carbonizable substances. After heating in a water bath for 2 minutes, remove the tube from the water bath, and immediately shake vigorously and vertically for 5 seconds. Repeat this procedure four times: the Liquid Paraffin layer remains unchanged in color, and the sulfuric acid layer has no more color than the following control solution.

Control solution: Mix 3.0 mL of Iron (III) Chloride CS...
with 1.5 mL of Cobalt (II) Chloride CS and 0.50 mL of Copper (II) Sulfate CS.

Containers and storage

Containers—Tight containers.

Light Liquid Paraffin

Light Liquid Paraffin is a mixture of liquid hydrocarbons obtained from petroleum.

Tocopherols of a suitable form may be added at a concentration not exceeding 0.001% as a stabilizer.

Description

Light Liquid Paraffin is a clear, colorless oily liquid, nearly free from fluorescence. It is odorless and tasteless.

It is freely soluble in diethyl ether, and practically insoluble in water and in ethanol (95%).

Boiling point: above 300°C.

Identification

(1) Heat Light Liquid Paraffin strongly in a porcelain dish, and fire: it burns with a bright flame and the odor of paraffin vapor is perceptible.

(2) Heat 0.5 of Light Liquid Paraffin with 0.5 g of sulfur with shaking carefully: the odor of hydrogen sulfide is perceptible.

Specific gravity $<2.560$, $\rho$: 0.830 – 0.870

Viscosity $<2.530$: Less than 37 mm²/s (Method 1, 37.8°C).

Purity

(1) Odor—Transfer a suitable amount of Light Liquid Paraffin to a small beaker, and heat on a water bath: no foreign odor is perceptible.

(2) Acidity or alkalinity—Shake vigorously 10 mL of Light Liquid Paraffin with 10 mL of hot water and 1 drop of phenolphthalein TS: no red color develops. Shake this solution with 0.20 mL of 0.02 mol/L sodium hydroxide VS: a red color develops.

(3) Heavy metals $<1.070$: Ignite 2.0 g of Light Liquid Paraffin in a crucible, first moderately until charred, then between 450°C and 550°C to ash. Cool, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. To the residue add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(4) Arsenic $<1.110$: Prepare the test solution with 1.0 g of Light Liquid Paraffin according to Method 3, and perform the test (not more than 2 ppm).

(5) Solid paraffin—Transfer 50 mL of Light Liquid Paraffin, previously dried at 105°C for 2 hours, to a Nessler tube, and cool in ice water for 4 hours: the turbidity produced, if any, is not deeper than that of the following control solution.

Control solution: To 1.5 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, add 1 mL of silver nitrate TS, and allow to stand for 5 minutes.

(6) Sulfur compounds—Prepare a saturated solution of lead (II) oxide in a solution of sodium hydroxide (1 in 5), and mix 2 drops of this clear solution with 4.0 mL of Light Liquid Paraffin and 2 mL of ethanol (99.5). Heat at 70°C for 10 minutes with frequent shaking, and cool: no dark brown color develops.

(7) Polycyclic aromatic hydrocarbons—Take 25 mL of Light Liquid Paraffin by a 25-mL measuring cylinder, transfer to a 100-mL separator, and wash out the cylinder with 25 mL of hexane for ultraviolet-visible spectrophotometry. Combine the washings with the liquid in the separator, and shake vigorously. Shake this solution vigorously for 2 minutes with 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and allow to stand for 15 minutes. Transfer the lower layer to a 50-mL separator, add 2 mL of hexane for ultraviolet-visible spectrophotometry, shake vigorously for 2 minutes, and allow to stand for 2 minutes. Transfer the lower layer to a glass-stoppered 10-mL centrifuge tube, and centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 15 minutes, and use the clear solution so obtained as the sample solution. Separately, transfer 25 mL of hexane for ultraviolet-visible spectrophotometry to a 50-mL separator, add 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, shake vigorously for 2 minutes, and allow to stand for 2 minutes. Transfer the lower layer to a glass-stoppered 10-mL centrifuge tube, centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution so obtained as a control solution. Immediately determine the absorbance of the sample solution using the control solution as the blank as directed under Ultraviolet-visible Spectrophotometry $<2.240$: not more than 0.10 at the wavelength region between 260 nm and 350 nm.

(8) Readily carbonizable substances—Transfer 5 mL of Light Liquid Paraffin to a Nessler tube, and add 5 mL of sulfuric acid for readily carbonizable substances. After heating in a water bath for 2 minutes, remove the tube from the water bath, and immediately shake vigorously and vertically for 5 seconds. Repeat this procedure four times: the liquid paraffin layer remains unchanged in color, and sulfuric acid layer has no more color than the following control solution.

Control solution: Mix 3.0 mL of Iron (III) Chloride CS with 1.5 mL of Cobalt (II) Chloride CS and 0.50 mL of Copper (II) Sulfate CS.

Containers and storage

Containers—Tight containers.

Paraformaldehyde

Paraformaldehyde contains not less than 95.0% of CH₂O: 30.03.

Description

Paraformaldehyde occurs as a white powder. It has a slight odor of formaldehyde, but a very strong irritating odor is perceptible when it is heated.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in hot water, in hot dilute hydrochloric acid, in
titrate stopper immediately, allow to stand for 15 minutes, and for 5 minutes. Then add 5 mL of dilute hydrochloric acid, 50 mL of 0.05 mol/L iodine VS, stopper, and allow to stand iodine flask. Add 40 mL of water and an exactly measured accurately weighed, in 10 mL of potassium hydroxide TS in an Assay Dissolve about 50 mg of Paraformaldehyde, accu-

Containers and storage Containers—Tight containers.

Identification (1) Dissolve 0.1 g of Paraformaldehyde in 5 mL of ammonia TS, add 5 mL of silver nitrate TS, shake, and add 3 mL of a solution of sodium hydroxide (1 in 10): a mirror of metallic silver is immediately formed on the sides of the container.

(2) Add a solution of 0.04 g of salicylic acid in 5 mL of sulfuric acid to 0.02 g of Paraformaldehyde, and warm slowly: a persistent, dark red color is produced.

Purity (1) Clarity and color of solution—Dissolve 0.20 g of Paraformaldehyde in 10 mL of ammonia TS: the solution is clear and colorless.

(2) Acidity or alkalinity—To 0.5 g of Paraformaldehyde add 10 mL of water, shake vigorously for 1 minute, and filter: the filtrate is neutral.

(3) Chloride <1.03>#Dissolve 1.5 g of Paraformaldehyde in 75 mL of water and 7.5 mL of sodium carbonate TS, evaporate on a water bath to dryness, and ignite at about 500°C. Dissolve the residue in 15 mL of water, filter, if necessary, neutralize with diluted nitric acid (3 in 10), and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 7.5 mL of sodium carbonate TS, a volume of diluted nitric acid (3 in 10) required for neutralization of the sample, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.006%).

(4) Sulfate <1.14>—Dissolve 1.5 g of Paraformaldehyde in 45 mL of water and 4.5 mL of sodium carbonate TS, evaporate on a water bath to dryness, and ignite at about 500°C. Dissolve the residue in 15 mL of water, filter, if necessary, neutralize the diluted hydrochloric acid (3 in 5), and boil for 5 minutes. After cooling, add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 4.5 mL of sodium carbonate TS add an equal volume of diluted hydrochloric acid (3 in 5) for the neutralization of the sample and 15 mL of water, and boil for 5 minutes. After cooling, add 0.35 mL of 0.005 mol/L sulfuric acid VS, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.011%).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Dissolve about 50 mg of Paraformaldehyde, accurately weighed, in 10 mL of potassium hydroxide TS in an iodine flask. Add 40 mL of water and an exactly measured 50 mL of 0.05 mol/L iodine VS, stopper, and allow to stand for 5 minutes. Then add 5 mL of dilute hydrochloric acid, stopper immediately, allow to stand for 15 minutes, and titrate <2.30>: the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS = 1.501 mg of CH₂O

Containers and storage Containers—Tight containers.

Dental Paraformaldehyde Paste

歯科用パラホルムパスタ

Method of preparation

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraformaldehyde, finely powdered</td>
<td>35 g</td>
</tr>
<tr>
<td>Procaine Hydrochloride, finely powdered</td>
<td>35 g</td>
</tr>
<tr>
<td>Hydrous Lanolin</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 100 g

Prepare as directed under Ointments, with the above ingredients.

Description Dental Paraformaldehyde Paste is yellowish white in color. It has a characteristic odor.

Identification (1) To 0.15 g of Dental Paraformaldehyde Paste add 20 mL of diethyl ether and 20 mL of 0.5 mol/L sodium hydroxide TS, shake well, separate the water layer, and dilute with water to make 100 mL. To 1 mL of this solution add 10 mL of acetylacetone TS, and heat on a water bath for 10 minutes: a yellow color is produced (paraformaldehyde).

(2) To the diethyl ether layer obtained in (1) add 5 mL of dilute hydrochloric acid and 20 mL of water, shake well, and separate the water layer: the solution responds to Qualitative Tests <1.09> for primary aromatic amines (procaine hydrochloride).

(3) To 0.15 g of Dental Paraformaldehyde Paste add 25 mL of diethyl ether and 25 mL of water, shake, separate the water layer, filter, and use the filtrate as the sample solution. Separately, dissolve 0.01 g of procaine hydrochloride in 5 mL of water, and use this solution as standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia solution (28) (50:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): spots from the sample solution and standard solution show the same Rf value.

Containers and storage Containers—Tight containers.
Parnaparin Sodium

パルナパリンナトリウム

Parnaparin Sodium is a low-molecular heparin sodium obtained by depolymerization, with hydrogen peroxide and with copper (II) acetate, of heparins sodium from the healthy edible porcine intestinal mucosa. The mass-average molecular mass ranges between 4500 and 6400.

The potency is not less than 70 low-molecular-mass-heparin units and not more than 95 low-molecular-mass-heparin units of anti-factor Xa activity per milligram calculated with reference of the dried substance.

Description

Parnaparin Sodium occurs as a white or light yellow powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Identification

(1) Mix 0.1 mL of a solution of Parnaparin Sodium (1 in 20) and 10 mL of a solution of tritoluidine blue O (1 in 100,000), and shake the mixture: the blue color of solution immediately changes to purple.

(2) A solution of Parnaparin Sodium (1 in 20) responds to Qualitative Tests for sodium salt.

pH

Dissolve 0.1 g of Parnaparin Sodium in 10 mL of water: the pH of this solution is between 6.0 and 8.0.

Purity

(1) Clarity and color of solution—Dissolve 1.0 g of Parnaparin Sodium in 10 mL of water: the solution is clear and colorless or pale yellow.

(2) Heavy metals—Proceed with 1.0 g of Parnaparin Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying

Not more than 8.0% (0.2 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Molecular mass

Calculate the molecular mass of Parnaparin Sodium by the following methods: The mass-average molecular mass ranges between 4500 and 6400.

(i) Creation of calibration curve—Weigh 20 mg of low-molecular mass heparin for calibration of molecular mass, and dissolve it in 2.0 mL of the mobile phase as the standard solution. Perform the test with 50 μL of the standard solution as directed under Liquid Chromatography according to the following conditions. Determine the peak height, $H_{UV}$, in chromatogram obtained by the ultraviolet absorption photometer, and determine the peak height, $H_R$, in chromatogram obtained by the differential refractometer. Calculate the ratio of $H_{UV}$ to $H_R$, $H_{UV}/H_R$, at each peak. Assume the molecular mass in the 4th peak from the low molecular mass in chromatogram obtained by the ultraviolet absorption photometer as 2400, and make the calculation of the standard coefficient from dividing 2400 by the $H_{UV}/H_R$ at the corresponding peak. Make the calculation to multiply the $H_{UV}/H_R$ at each peak by the standard coefficient, and determine the molecular mass of each peak by the calculation. Prepare the calculation curve by plotting the logarithm of molecular masses at each peak on the vertical axis and the retention time on the chromatogram obtained by the differential refractometer on the horizontal axis.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 234 nm) and a differential refractometer.

Column: Connect two stainless steel columns which are 7.5 mm in inside diameter and 30 cm in length, and are packed with porous silica gel for liquid chromatography; one column, the molecular mass of limited size exclusion is about 500,000; the other, the molecular mass of limited size exclusion is about 100,000. Connect a pump, the about 500,000-molecular mass of limited size exclusion column, the about 100,000-molecular mass of limited size exclusion column, the ultraviolet absorption photometer and the differential refractometer in this order.

Column temperature; A constant temperature of about 40°C.

Mobile phase: Dissolve 28.4 g of sodium sulfate anhydride in 1000 mL of water, and 5.0 with 0.05 mol/L sulfuric acid TS.

Flow rate: 0.5 mL per minute.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, confirm that more than ten peaks in chromatogram obtained as directed under either the Ultraviolet-visible Spectrophotometry, or the Differential Refractometry are observed.

System repeatability: When the tests repeated 6 times with 50 μL of the standard solution under the above operating conditions, relative standard deviation of the 4th peak height in chromatogram ($H_{UV}$ and $H_R$) is not more than 3.0%.

(ii) Determination of molecular mass—Dissolve the 20 mg of Parnaparin Sodium with 2.0 mL of mobile phase, and use this solution as the sample solution. Perform the test with 50 μL of the sample solution as directed under Liquid Chromatography according to the following conditions. Divide the main peak observed between 30 min and 45 min to 30 sec-interval fractions, and determine the strength of differential refractometer of each 30 sec-interval fraction. Determine the molecular mass of each fraction using the calibration curve and the retention time of each fraction. Determine the mean of molecular mass in the entire peak using the strength of differential refractometer and the molecular mass in every fractions.

Mean molecular mass of parnaparin sodium

$$
\bar{M} = \left( \sum n_i \cdot M_i \right) / \sum n_i
$$
n: The differential refractometer strength of fraction i in the main peak of chromatogram

Mf: Molecular mass of fraction i in main peak

Operating conditions—
Detector: A differential refractometer.
Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in (i) Creation of calibration curve.
System suitability—
Proceed as directed in (i) Creation of calibration curve.

Distribution of molecular mass The molecular mass of Parnaparin Sodium is calculated as directed in the determination of molecular mass and the distribution of molecular mass is calculated by the following equation: the molecular mass of not less than 80% parnaparin sodium is between 1500 and 10,000.

Distribution of molecular mass (%)
\[ = \left( \frac{\Sigma n_i}{\Sigma n} \right) \times 100 \]

n: The differential refractometer strength of fraction i in the main peak of chromatogram

\( \Sigma n_i \): Sum of differential refractometer strength in the each fraction between 1500 and 10,000 molecular mass in the main peak

The degree of sulfate ester Dissolve 0.5 g of Parnaparin Sodium with 10 mL water. Treat the solution with 5 mL of a strongly basic ion exchange resin, and subsequently with 10 mL of a strongly acidic ion exchange resin. Dilute the solution with water to 50 mL, and titrate with 0.1 mol/L Sodium hydroxide VS (potentiometric titration). Calculate the degree of sulfate ester of Parnaparin Sodium from the equivalence point by the following equation; it is between 2.0 and 2.4.

The degree of sulfate ester
\[ = \frac{\text{the first equivalence point (mL)}}{\text{the second equivalence point (mL)}} \times [\text{first equivalence point (mL)}] \]

Total nitrogen Weigh accurately about 0.10 g of Parnaparin Sodium which is dried, and perform the test as directed under Nitrogen Determination (<1.0%) by potentiometric titration. It contains not less than 1.9% and not more than 2.3% of nitrogen (N: 14.01).

Anti-factor IIa activity Determine the potency of anti-factor IIa activity of Parnaparin Sodium according to the following method, it contains not less than 35 and not more than 60 low-molecular-mass-heparin unit per milligram calculated with reference to the dried substance.

(i) Standard solution Dissolve Low-molecular Mass Heparin RS in isotonic sodium chloride solution to make solutions which contain 0.4, 0.6 and 0.8 low-molecular-mass-heparin units (anti-factor IIa activity) in 1 mL, respectively.

(ii) Sample solution Weigh accurately about 50 mg of Parnaparin Sodium, and dissolve it with isotonic sodium chloride solution to adjust the solution which contains 4 μg parnaparin sodium in 1 mL.

(iii) Procedure To each plastic tube add 0.10 mL of the sample solution and the standard solution, separately. To each tube add 0.10 mL of human normal plasma and mix, and incubate at 37 ± 1°C accurately for 1 minute. Next, to each test tube add 0.10 mL of activated thromboplastin-time assay solution, which is pre-warmed at 37 ± 1°C, and after the mixing incubate accurately for 5 minutes at 37 ± 1°C. Then, to each tube add 0.10 mL of sodium calcium solution (277 in 100,000) which is pre-warmed at 37 ± 1°C, mix, start a stop watch simultaneously, and permit to stand at the same temperature. Determine the time for the first appearance of fibrin clot.

(iv) Calculation Determine the low-molecular-mass-heparin unit (anti-factor IIa activity) of the sample solution from calibration curve obtained plots of clotting times for each standard solution; calculate the low-molecular-mass-heparin unit (anti-factor IIa activity) for 1 mg of parnaparin sodium as following equation.

The low-molecular-mass-heparin unit (anti-factor IIa activity) for 1 mg of parnaparin sodium = the low-molecular-mass-heparin unit (anti-factor IIa activity) in 1 mL of sample solution \[ \times \frac{a}{b} \]

a: Amount (mg) of Parnaparin Sodium
b: The total volume (mL) in which Parnaparin Sodium has been dissolved with isotonic sodium chloride solution for the preparation of sample solution

The ratio of anti-factor Xa activity to anti-factor IIa activity Divide the anti-factor Xa activity, obtained in the Assay, by the anti-factor IIa activity which has been obtained from the test according to the method of anti-factor IIa activity; the ratio of anti-factor Xa activity to anti-factor IIa activity is between 1.5 and 2.5.

Assay

(i) Standard solution Dissolve Low-molecular Mass Heparin RS in isotonic sodium chloride solution to make solutions which contain 0.4, 0.6 and 0.8 low-molecular-mass-heparin units (anti-factor Xa activity) in 1 mL, respectively.

(ii) Sample solution Weigh accurately about 50 mg of Parnaparin Sodium, and dissolve it in isotonic sodium chloride solution to make a solution which contains 7 μg parnaparin sodium in 1 mL.

(iii) Procedure To each plastic tube add 0.10 mL of the sample solution or the standard solution, separately. Subsequently to the every tubes add 0.70 mL of Tris-buffered solution (pH 8.4), 0.10 mL of anti-thrombin III TS, and 0.10 mL of normal human plasma, and mix them. To another plastic tube transfer 0.20 mL of these solutions, separately, and incubate for accurate 3 minutes at 37 ± 1°C. Next, to each tube add 0.20 mL of chromogenic synthetic substrate solution (3 in 4000) and mix it, and subsequently incubate accurately for 3 minutes at 37 ± 1°C, and immediately add 0.10 mL of chromogenic synthetic substrate solution (3 in 4000) and mix it, and subsequently incubate accurately for 3 minutes at 37 ± 1°C. To each test tube add 0.30 mL of diluted acetic acid (100) solution (1 in 2) to stop the reaction. Separately, to plastic tube add 0.10 mL of isotonic sodium chloride solution, 0.70 mL of Tris-buffered solution (pH 8.4), 0.10 mL of anti-thrombin III TS, and 0.10 mL of normal human plasma to every tubes, and mix well. To another plastic tube transfer 0.20 mL of the solution, separately, and add both 0.30 mL of water and 0.30 mL of diluted acetic acid (100) (1 in 2). Determine the absorbance of both the sample solution and the standard solution at 405 nm as directed under Ultraviolet-visible Spectrophotometry (<2.4%) using a solution obtained from this solution as the blank.

(iv) Calculation method Determine the low-molecular-mass unit (anti-factor Xa activity) of the sample solution
using the calibration curve prepared from the absorbance of the standard solutions and their logarithmic concentrations, and calculate the low-molecular-mass unit (anti-factor Xa activity) in 1 mg of Parnaparin Sodium.

Low-molecular-mass-heparin unit (anti-factor Xa activity) in 1 mg of Parnaparin Sodium

\[ \text{unit} = \frac{b}{a} \]

\( a \): Amount (mg) of Parnaparin Sodium
\( b \): The total volume (mL) in which Parnaparin Sodium has been dissolved with isotonic sodium chloride solution for the preparation of sample solution

**Container and Storage**

Containers—Well-closed containers.

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**Peanut Oil**

*Oleum Arachidis*

ラッカセイ油

Peanut Oil is the fixed oil obtained from the seeds of *Arachis hypogaea* Linné (*Leguminosae*).

**Description**

Peanut Oil is a pale yellow, clear oil. It is odorless or has a slight odor. It has a mild taste.

It is miscible with diethyl ether and with petroleum ether.

It is slightly soluble in ethanol (95%).

Specific gravity: 0.909 – 0.916

Congealing point of the fatty acids: 22 – 33°C

**Identification**

Saponify 5 g of Peanut Oil by boiling with 2.5 mL of sodium hydroxide solution (3 in 10) and 12.5 mL of ethanol (95). Evaporate the ethanol, dissolve the residue in 50 mL of hot water, and add dilute hydrochloric acid in excess until the free fatty acids separate as an oily layer. Cool the mixture, remove the separated fatty acids, and dissolve them in 75 mL of diethyl ether. To the diethyl ether solution add a solution of 4 g of lead (II) acetate trihydrate in 40 mL of ethanol (95), and allow the mixture to stand for 18 hours. Filter the supernatant liquid, transfer the precipitate to the filter with the aid of diethyl ether, and filter by suction. Place the precipitate in a beaker, heat it with 40 mL of dilute hydrochloric acid and 20 mL of water until the oily layer is entirely clear, cool, and decant the water layer. Boil the fatty acids with 50 mL of dilute hydrochloric acid (1 in 100). When the solution prepared by dissolving 0.1 g of the dried crystals is between 73°C and 76°C.

**Acid value** <1.13> Not more than 0.2.

**Saponification value** <1.13> 188 – 196

**Unsaponifiable matters** <1.13> Not more than 1.5%

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**Pemirolast Potassium**

ペミロラストカリウム

C_{9}H_{7}K_{6}O: 266.30

Monopotassium 5-(9-methyl-4-oxo-4H-pyrido[1,2-α]pyrimidin-3-yl)-1H-tetrazol-1-ide

[100299-08-9]

Pemirolast Potassium contains not less than 98.5% and not more than 101.0% of C_{9}H_{7}K_{6}O, calculated on the anhydrous basis.

**Description**

Pemirolast Potassium occurs as a light yellow crystalline powder.

It is freely soluble in water, slightly soluble in methanol, and very slightly soluble in ethanol (99.5).

It dissolves in potassium hydroxide TS.

Melting point: about 322°C (with decomposition).

**Identification**

(1) Determine the absorption spectrum of a solution of Pemirolast Potassium in diluted potassium hydroxide TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pemirolast Potassium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pemirolast Potassium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Pemirolast Potassium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Pemirolast Potassium responds to the Qualitative Tests <1.09> (1) for potassium salt.

**Purity**

(1) Clarity and color of solution—A solution obtained by dissolving 0.5 g of Pemirolast Potassium in 10 mL of water is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 0.5 g of Pemirolast Potassium according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 50 mg of Pemirolast Potassium in 50 mL of a mixture of phosphate buffer solution, pH 8.0 and methanol (3:2), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add a mixture of phosphate buffer solution, pH 8.0 and methanol (3:2) to make exactly 100 mL. To exactly 2.5 mL of this solution add a mixture of phosphate buffer solution, pH 8.0 and methanol (3:2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10μL of each of the sample solution and standard solution as
directed under Liquid Chromatography according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than pemirolast obtained from the sample solution is not larger than the peak area of pemirolast from the standard solution.

**Operating conditions—**

Detector: column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 9 times as long as the retention time of pemirolast.

**System suitability—**

Test for required detectability: To exactly 5 mL of the standard solution add a mixture of phosphate buffer solution, pH 8.0 and methanol (3:2) to make exactly 25 mL. Confirm that the peak area of pemirolast obtained with 10 μL of this solution is equivalent to 15 to 25% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pemirolast is not more than 2.0%.

(4) Residual solvent—Being specified separately.

**Water**  Not more than 0.5% (0.1 g, coulometric titration).

**Assay** Weigh accurately about 50 mg each of Pemirolast Potassium and Pemirolast Potassium RS (separately determine the water in the same manner as Pemirolast Potassium), dissolve in a mixture of phosphate buffer solution, pH 8.0 and methanol (3:2) to make them exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution to each, then add a mixture of phosphate buffer solution, pH 8.0 and methanol (3:2) to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q₁ and Q₅, of the peak area of pemirolast to that of the internal standard.

Amount (mg) of C₁₀H₇KN₆O₅ = Mₛ × Q₁/Q₅

Mₛ: Amount (mg) of Pemirolast Potassium RS, calculated on the anhydrous basis

**Internal standard solution—** A solution of ethyl aminobenzoate in methanol (1 in 1000).

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadeucylsilylized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, methanol and acetic acid (100) (30:20:1).

Flow rate: Adjust the flow rate so that the retention time of pemirolast is about 5 minutes.

**System suitability—**

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, pemirolast and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pemirolast to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

**Pemirolast Potassium for Syrup**

シロップ用ベミロラストカリウム

Pemirolast Potassium for Syrup is a preparation for syrup, which is dissolved before use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of pemirolast potassium (C₁₀H₇KN₆O: 266.30).

**Method of preparation** Prepare as directed under Preparations for Syrups, with Pemirolast Potassium.

**Identification** Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry. It exhibits maxima between 255 nm and 259 nm and between 355 nm and 359 nm.

**pH** Being specified separately.

**Uniformity of dosage units** Perform the test according to the following method: Pemirolast Potassium for Syrup in single-unit containers meet the requirement of the Content uniformity test.

Dissolve the total amount of the content of 1 container of Pemirolast Potassium for Syrup in water to make exactly 10 mL so that each mL contains about 50 μg of pemirolast potassium (C₁₀H₇KN₆O). Pipet 10 mL of this solution, add water to make exactly 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of pemirolast potassium (C₁₀H₇KN₆O) = Mₛ × A₁/A₅ × V/400

Mₛ: Amount (mg) of Pemirolast Potassium RS, calculated on the anhydrous basis

**Assay** Powder Pemirolast Potassium for Syrup. Weigh accurately a portion of the powder, equivalent to about 5 mg of pemirolast potassium (C₁₀H₇KN₆O), and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Pemirolast Potassium RS (separately determine the water in the same manner as Pemirolast Potassium), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this
solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_t \) and \( A_s \), at 357 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\).  

\[
\text{Amount (mg) of pemirolast potassium (C}_{10}H_7KN_6O) = M_S \times \frac{A_t}{A_s} \times \frac{1}{4}
\]

\( M_S \): Amount (mg) of Pemirolast Potassium RS, calculated on the anhydrous basis

Containers and storage  Containers—Tight containers. Storage—Light-resistant.

**Pemirolast Potassium Tablets**

ベミロラストカリウム錠

Pemirolast Potassium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of pemirolast potassium (C\(_{10}\)H\(_7\)KN\(_6\)O: 266.30).

**Method of preparation**  Prepare as directed under Tablets, with Pemirolast Potassium.

**Identification**  Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry \(<2.24\): it exhibits maxima between 255 nm and 259 nm, and between 355 nm and 359 nm.

**Uniformity of dosage units \(<6.02\)**  Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Pemirolast Potassium Tablets add 50 mL of water for 5 mg of pemirolast potassium (C\(_{10}\)H\(_7\)KN\(_6\)O), and shake to disintegrate the tablet completely. Then, add water to make exactly \( V \) mL so that each mL contains about 50 \( \mu \)g of pemirolast potassium (C\(_{10}\)H\(_7\)KN\(_6\)O), and filter. Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add 1 mL of diluted potassium hydroxide TS (1 in 100), and filter through a membrane filter with a pore size not exceeding 0.45 \( \mu \)m. Discard the first 10 mL of the filtrate, pipet \( V' \) mL of the subsequent filtrate, and add the dissolution medium to make exactly \( V' \) mL so that each mL contains about 5.6 \( \mu \)g of pemirolast potassium (C\(_{10}\)H\(_7\)KN\(_6\)O) according to the labeled amount. Pipet 4 mL of this solution, add exactly 2 mL of diluted potassium hydroxide TS (1 in 10), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Pemirolast Potassium RS (separately determine the water \(<2.48\) in the same manner as Pemirolast Potassium), dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 25 mL. Pipet 4 mL of this solution, add exactly 2 mL of diluted potassium hydroxide TS (1 in 10), and use this solution as the standard solution. Then, proceed as directed in the Assay.

Dissolution rate (\%) with respect to the labeled amount of pemirolast potassium (C\(_{10}\)H\(_7\)KN\(_6\)O)  
\[
\frac{M_S \times A_t/A_s \times V'/V \times 1/C \times 18}{M_S} = \frac{M_S \times A_t/A_s \times V'/V \times 1/C \times 18}{M_S}
\]

\( M_S \): Amount (mg) of Pemirolast Potassium RS, calculated on the anhydrous basis

**Assay**  Accurately weigh the mass of not less than 20 Pemirolast Potassium Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of pemirolast potassium (C\(_{10}\)H\(_7\)KN\(_6\)O), add 50 mL of water, shake thoroughly for 20 minutes, then add water to make exactly 100 mL. Filter, discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add 1 mL of diluted potassium hydroxide TS (1 in 100), add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Pemirolast Potassium RS (separately determine the water \(<2.48\) in the same manner as Pemirolast Potassium), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add 1 mL of diluted potassium hydroxide TS (1 in 100), add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, \( A_t \) and \( A_s \), at 357 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24\>, using water as the blank.

\[
\text{Amount (mg) of pemirolast potassium (C}_{10}H_7KN_6O) = M_S \times A_t/A_s \times V'/V \times 1/C \times 18
\]

\( M_S \): Amount (mg) of Pemirolast Potassium RS, calculated on the anhydrous basis

Containers and storage  Containers—Tight containers. Storage—Light-resistant.
Penbutolol Sulfate

ペンプトロール硫酸塩

\[
\text{(C}_{18}\text{H}_{29}\text{NO}_2)_2\text{H}_2\text{SO}_4; 680.94}
\]

Penbutolol Sulfate, when dried, contains not less than 98.5% of (C\(_{18}\)H\(_{29}\)NO\(_2\))\(_2\)H\(_2\)SO\(_4\).

**Description**  Penbutolol Sulfate occurs as a white crystalline powder.

It is very soluble in acetic acid (100), freely soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in water, and practically insoluble in acetic anhydride and in diethyl ether.

**Identification (1)**  Determine the absorption spectrum of a solution of Penbutolol Sulfate in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Penbutolol Sulfate, previously dried, as directed in the paste method under Infrared Spectrophotometry \(<2.25>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.1 g of Penbutolol Sulfate in 25 mL of water by warming, and cool: this solution responds to Qualitative Tests \(<1.00>\) for sulfate.

**Optical rotation** \(<2.49>\)  \([\alpha]_D^20\) = \(-23\) to \(-25^\circ\) (after drying, 0.2 g, methanol, 20 mL, 100 mm).

**Melting point** \(<2.69>\)  213 – 217°C

**Purity (1)**  Heavy metals \(<1.07>\)—Proceed with 2.0 g of Penbutolol Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic \(<1.11>\)—Prepare the test solution with 1.0 g of Penbutolol Sulfate according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.8 g of Penbutolol Sulfate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Prepare the test with these solutions as directed under Thin-layer Chromatography \(<2.05>\). Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, ethanol (95) and ammonia solution (28) (85:12:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** \(<2.41>\)  Not more than 0.5% (0.5 g, 105°C, 3 hours).

**Residue on ignition** \(<2.44>\)  Not more than 0.2% (1 g).

**Assay**  Weigh accurately about 0.8 g of Penbutolol Sulfate, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate \(<2.50>\) with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 68.09 mg of (C\(_{18}\)H\(_{29}\)NO\(_2\))\(_2\)H\(_2\)SO\(_4\).

**Containers and storage**  Containers—Well-closed containers.

Pentazocine

ペンタゾシン

C\(_{19}\)H\(_{27}\)NO: 285.42

\((2RS,6RS,11RS)-6,11\text{-Dimethyl-3-(3-methylbut-2-en-1-yl)-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzoazocin-8-ol}\)

Pentazocine, when dried, contains not less than 99.0% of C\(_{19}\)H\(_{27}\)NO.

**Description**  Pentazocine occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in acetic acid (100) and in chloroform, soluble in ethanol (95), sparingly soluble in diethyl ether and practically insoluble in water.

**Identification (1)**  To 1 mg of Pentazocine add 0.5 mL of formaldehyde-sulfuric acid TS: a deep red color is produced, and it changes to grayish brown immediately.

(2) Dissolve 5 mg of Pentazocine in 5 mL of sulfuric acid, add 1 drop of iron (III) chloride TS, and heat in a water bath for 2 minutes: the color of the solution changes from light yellow to deep yellow. Shake the solution with 1 drop of nitric acid: the solution remains yellow in color.

(3) Determine the absorption spectrum of a solution of Pentazocine in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Absorbance** \(<2.24>\)  \(E_{1%c/m}^1\) (278 nm): 67.5 – 71.5 (after drying, 0.1 g, 0.01 mol/L hydrochloric acid TS, 1000 mL).
It is sparingly soluble in water, slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

A solution of Pentobarbital Calcium (1 in 100) shows no optical rotation.

**Identification (1)** Determine the infrared absorption spectrum of Pentobarbital Calcium as directed in the potassium bromide disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) To 1 g of Pentobarbital Calcium add 5 mL of ethanol (95) and 5 mL of dilute hydrochloric acid, dissolve by warming with shaking, shake with 5 mL of dilute hydrochloric acid and 10 mL of water, allow to cool, and filter. To the filtrate add 1 drop of methyl red TS, and add ammonia TS until a slight yellow color develops: the solution responds to Qualitative Tests <1.09>(1), (2) and (3) for calcium salt.

**Purity (1)** Chloride <1.03>—To 1.0 g of Pentobarbital Calcium add 5 mL of ethanol (95) and 2.5 mL of dilute nitric acid, dissolve by warming with shaking, cool, add water to make 50 mL, shake well, and filter. Discard the first 10 mL of the filtrate, and to 15 mL of the subsequent filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 1.5 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.035%).

(2) Heavy metals <1.07>—To 2.0 g of Pentobarbital Calcium add 5 mL of ethanol (95) and 5 mL of dilute hydrochloric acid, dissolve by warming with shaking, cool, add water to make 50 mL, shake well, and filter. Discard the first 10 mL of the filtrate, to 10 mL of the subsequent filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 2.5 mL of ethanol (95) add 2.5 mL of dilute hydrochloric acid and water to make 30 mL. Add 1 drop of phenolphthalein TS, add dropwise ammonia TS until a pale red color develops, then add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 2.5 mL of ethanol (95) add 2.5 mL of dilute hydrochloric acid and water to make 30 mL. Add 1 drop of phenolphthalein TS, add dropwise ammonia TS until a pale red color develops, then add 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(3) Related substances—Dissolve 10 mg of Pentobarbital Calcium in 100 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the areas of each peak by the automatic integration method: the area of any peak other than the peak of pentobarbital from the sample solution is not larger than 3/10 times the peak area of pentobarbital from the standard solution, and the total of these peak areas is not larger than the peak area of pentobarbital from the standard solution.

**Operating conditions**—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the

**Melting point** 150 – 158°C

**Purity (1)** Clarity and color of solution—Dissolve 0.10 g of Pentobarbital in 10 mL of acetic acid (100), and titrate the solution with exactly 20 mL of 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 28.54 mg of C19H27NO₃.

**Residue on ignition** <2.44>—Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.5 g of Pentazocine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate the solution with exactly 20 mL of 0.1 mol/L perchloric acid VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 15.27 mg of C22H34CaN4O6.

**Loss on drying** <2.47>—Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 5 hours).

**Residue on ignition** <2.44>—Not more than 0.2% (1 g).

**Related substances**—Dissolve 0.20 g of Pentazocine in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and isopropylamine (94:3:3) to a distance of about 13 cm, and air-dry the plate. Allow to stand for 5 minutes in iodine vapor: any spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

**Containers and storage** Containers—Well-closed containers.

**Pentobarbital Calcium**

ペントバルビタールカルシウム

C₂₂H₃₄CaN₄O₆: 490.61
Monoclinic bix[5-ethyl-5-{(1RS)-1-methylbutyl]-4,6-dioxo-1,4,5,6-tetrahydroprymidin-2-olate]
[76-74-4, Pentobarbital]

Pentobarbital Calcium contains not less than 98.0% and not more than 102.0% of C₂₂H₃₄CaN₄O₆, calculated on the dried basis.

**Description** Pentobarbital Calcium occurs as a white powder.
retention time of pentobarbital beginning after the solvent peak.

**System suitability—**

Test for required detection: Pipet 2 mL of the standard solution, add water to make exactly 20 mL, and confirm that the peak area of pentobarbital obtained from 20 μL of this solution is equivalent to 5 to 15 times the area of pentobarbital from 20 μL of the standard solution.

System performance: Proceed as directed in the system performance in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of pentobarbital is not more than 5%.

**Loss on drying <2.4>** Not more than 7.0% (1 g, 105°C, 5 hours).

**Assay** Weigh accurately about 20 mg of Pentobarbital Calcium, dissolve in 5 mL of water, add exactly 5 mL of the internal standard solution and water to make 20 mL. To 5 mL of this solution add water to make 20 mL. To 2 mL of this solution add water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 18 mg of Pentobarbital RS, previously dried at 105°C for 2 hours, dissolve in 10 mL of acetonitrile, add exactly 5 mL of the internal standard solution and water to make 50 mL. To 5 mL of this solution add water to make 20 mL. To 2 mL of this solution add water to make 20 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of pentobarbital to that of the internal standard.

\[
\text{Amount (mg) of C}_{20}\text{H}_{31}\text{NO}_3\text{CaN}_2\text{O}_6 = M_S \times \frac{Q_T}{Q_S} \times 1.084
\]

$M_S$: Amount (mg) of Pentobarbital RS

**Internal standard solution**—Dissolve 0.2 g of isopropyl parahydroxybenzoate in 20 mL of acetonitrile, and add water to make 100 mL.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogenphosphate in 1000 mL of water, and adjust to pH 4.0 with diluted phosphoric acid (1 in 10). To 650 mL of this solution add 350 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pentobarbital is about 7 minutes.

**System suitability—**

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, pentobarbital and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pentobarbital to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Well-closed containers.

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**Pentoxysterine Citrate**

**Carbetapentane Citrate**

**Carbetapentane Citrate**

Pentoxysterine Citrate, when dried, contains not less than 98.5% of C_{20}H_{31}NO_3\cdot C_6H_8O_7.

**Description** Pentoxysterine Citrate occurs as a white, crystalline powder. It is very soluble in acetic acid (100), freely soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

**Identification** (1) Dissolve 0.1 g of Pentoxysterine Citrate in 10 mL of water, and add 10 mL of Reinecke salt TS: a light red precipitate is formed.

(2) Determine the infrared absorption spectrum of Pentoxysterine Citrate, previously dried, as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Pentoxysterine Citrate (1 in 10) responds to Qualitative Tests <1.09> (1) and (2) for citrate.

**Melting point <2.6.0>** 92 – 95°C

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Pentoxysterine Citrate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Pentoxysterine Citrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Pentoxysterine Citrate according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Pentoxysterine Citrate in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 15 μL each of the sample solution and standard solution on a
Peplomycin Sulfate

**Description**

Peplomycin Sulfate is the sulfate of a substance having antitumor activity produced by the growth of *Streptomyces verticillus*.

It contains not less than 865 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the dried basis. The potency of Peplomycin Sulfate is expressed as mass (potency) of peplomycin monosulfate \(\text{C}_{41}\text{H}_{88}\text{N}_{18}\text{O}_{21}\text{S}_{2}\cdot\text{H}_{2}\text{SO}_{4}\) \(1571.67\) (0.1 g calculated on the dried basis, 0.1 mol/L phosphate buffer solution, pH 5.3, 10 mL, 100 mm).

**Purity (1)**

Clarity and color of solution—Dissolve 80 mg of Peplomycin Sulfate in 4 mL of water: the solution is clear and colorless.

**Gas**

Combustible gas—Acetylene.

**Supporting gas**—Air.

**Wavelength**—324.8 nm.

**Lamp**—Copper hollow cathode lamp.

**Operating conditions**—

Detector, column, column temperature, mobile phase stock solution, mobile phase A, mobile phase B, flow rate: Proceed as directed in the operating conditions in the Purity (3).

A solution of Peplomycin Sulfate (1 in 200) responds less than that of the standard solution (not more than 2.50 mAU at 70384-29-1) and not more than 1010 mAU (1 in 50) to a distance of about 10 cm, and air-dry the plate. Allow to stand in iodine vapor for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying**

Not more than 0.5% (1 g, in vacuum, phosphorus(V) oxide, 60°C, 4 hours).

**Residue on ignition**

Not more than 0.1% (1 g).

**Assay**

Weigh accurately about 0.5 g of Peptoxverine Citrate, previously dried, dissolve in 30 mL of acetic anhydride, and titrate with 0.1 mol/L of perchloric acid VS until the color of the solution changes from purple through blue-green to green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 52.56 mg of \(\text{C}_{20}\text{H}_{31}\text{NO}_{3}\cdot\text{C}_{6}\text{H}_{8}\text{O}_{7}\) monosulfate: 1571.67.

**Containers and storage**

Containers—Well-closed containers.

It is hygroscopic.

**Identification (1)**

To 4 mg of Peplomycin Sulfate add 5 μL of copper (II) sulfate TS, and dissolve in water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \(\angle 2.24\), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Peplomycin Sulfate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 10 mg each of Peplomycin Sulfate and Peplomycin Sulfate RS in 6 mL of water, add 0.5 mL of a solution of copper (II) sulfate pentahydrate (1 in 125), and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL of each of these solutions as directed under Liquid Chromatography \(\angle 2.01\) according to the following conditions: the retention time of the principal peak obtained from the sample solution is the same as that from the standard solution.

**Operating conditions**—

Detector, column, column temperature, mobile phase stock solution, mobile phase A, mobile phase B, flowing of the mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (3).

(3) A solution of Peplomycin Sulfate (1 in 200) responds less than that of the sample solution (not more than 2.5 mAU at 70384-29-1) and not more than 1010 mAU (1 in 50) to a distance of about 10 cm, and air-dry the plate of silica gel for thin-layer chromatography. Immediately after air-drying, develop the plate with a mixture of chloroform, methanol, ethyl acetate and ammonia solution (28:25:10:10:1) to a distance of about 10 cm, and air-dry the plate. Allow to stand in iodine vapor for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying**

Not more than 0.5% (1 g, in vacuum, phosphorus(V) oxide, 60°C, 4 hours).

**Residue on ignition**

Not more than 0.1% (1 g).

**Assay**

Weigh accurately about 0.5 g of Peptoxverine Citrate, previously dried, dissolve in 30 mL of acetic anhydride, and titrate with 0.1 mol/L of perchloric acid VS until the color of the solution changes from purple through blue-green to green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 52.56 mg of \(\text{C}_{20}\text{H}_{31}\text{NO}_{3}\cdot\text{C}_{6}\text{H}_{8}\text{O}_{7}\) monosulfate: 1571.67.

**Containers and storage**

Containers—Well-closed containers.

It is hygroscopic.

**Identification (1)**

To 4 mg of Peplomycin Sulfate add 5 μL of copper (II) sulfate TS, and dissolve in water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \(\angle 2.24\), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Peplomycin Sulfate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 10 mg each of Peplomycin Sulfate and Peplomycin Sulfate RS in 6 mL of water, add 0.5 mL of a solution of copper (II) sulfate pentahydrate (1 in 125), and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL of each of these solutions as directed under Liquid Chromatography \(\angle 2.01\) according to the following conditions: the retention time of the principal peak obtained from the sample solution is the same as that from the standard solution.

**Operating conditions**—

Detector, column, column temperature, mobile phase stock solution, mobile phase A, mobile phase B, flowing of the mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (3).

(3) A solution of Peplomycin Sulfate (1 in 200) responds less than that of the sample solution (not more than 2.5 mAU at 70384-29-1) and not more than 1010 mAU (1 in 50) to a distance of about 10 cm, and air-dry the plate of silica gel for thin-layer chromatography. Immediately after air-drying, develop the plate with a mixture of chloroform, methanol, ethyl acetate and ammonia solution (28:25:10:10:1) to a distance of about 10 cm, and air-dry the plate. Allow to stand in iodine vapor for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.
cin is not more than 7.0%.

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase stock solution: Dissolve 0.96 g of sodium 1-pentanesulfonate and 1.86 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 1000 mL of water and 5 mL of acetic acid (100), and adjust the pH to 4.3 with ammonia TS.

Mobile phase A: A mixture of mobile phase stock solution and methanol (9:1).

Mobile phase B: A mixture of mobile phase stock solution and methanol (3:2).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 60</td>
<td>100 → 0</td>
<td>0 → 100</td>
</tr>
<tr>
<td>60 – 75</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Flow rate: 1.2 mL per minute.

Time span of measurement: As long as 20 minutes after elution of peplomycin beginning after the peak of copper sulfate.

**System suitability**—
Test for required detectability: Measure exactly 1 mL of the sample solution, add water to make exactly 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add water to make exactly 10 mL. Confirm that the peak area of peplomycin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of peplomycin are not less than 30,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of peplomycin is not more than 2.0%.

**Loss on drying** <2.47> Not more than 3.0% (60 mg, in vacuum, phosphorus (V) oxide, 60°C, 3 hours). Handle the sample avoiding absorption of moisture.

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Mycobacterium smegmatis* ATCC 607

(ii) Agar media for base and seed layer, and for transferring test organism

<table>
<thead>
<tr>
<th>Arabic Medicine</th>
<th>Chinese Medicine</th>
<th>English Medicine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerin</td>
<td>Peplomycin Sulfate</td>
<td>Peplomycin Sulfate</td>
</tr>
<tr>
<td>10.0 g</td>
<td>Equivalent to about 20 mg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.</td>
<td></td>
</tr>
<tr>
<td>Peptone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.0 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat extract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.0 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium chloride</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.0 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.0 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000 mL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mix all the ingredients and adjust the pH of the solution with sodium hydroxide TS so that it will be 6.9 to 7.1 after sterilization.

(iii) Liquid medium for suspending test organism

<table>
<thead>
<tr>
<th>Arabic Medicine</th>
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<th>English Medicine</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
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</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>3.0 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000 mL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mix all the ingredients and adjust the pH of the solution with sodium hydroxide TS so that it will be 6.9 to 7.1 after sterilization.

(iv) Preparation of agar medium of seeded layer—Inoculate the test organism onto the slant of the agar medium for transferring test organism, and incubate the slant at 27°C for 40 to 48 hours. Inoculate the subcultured test organism into 100 mL of the liquid medium for suspending test organism, incubate at 25 to 27°C for 5 days while shaking, and use this suspension as the suspension of the test organism. Keep the suspension of the test organism at a temperature of not exceeding 5°C and use within 14 days. Add 0.5 mL of the suspension of the test organism in 100 mL of the Agar medium for seed layer previously kept at 48°C, mix thoroughly, and use this as the agar medium of seeded layer.

(v) Preparation of cylinder-agar plate—Proceed as directed in 1.7. Preparation of cylinder-agar plates with the exception of the amounts of the agar medium for base layer and the agar medium of seeded layer to put in the Petri dish, which are 5.0 mL and 8.0 mL, respectively.

(vi) Standard solutions—Weigh accurately an amount of Peplomycin Sulfate RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5°C, and use within 15 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 4 μg (potency) and 2 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(vii) Sample solutions—Weigh accurately an amount of Peplomycin Sulfate, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 4 μg (potency) and 2 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

**Containers and storage** Containers—Tight containers.
Peplomycin Sulfate for Injection

Peplomycin Sulfate for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 115.0% of the labeled amount of peplomycin (C_{61}H_{48}N_{13}O_{21}S_{2}: 1473.59).

Method of preparation Prepare as directed under Injections, with Peplomycin Sulfate.

Description Peplomycin Sulfate for Injection occurs as white light masses or powder.

Identification Take an amount of Peplomycin Sulfate for Injection, equivalent to 10 mg (potency) of Peplomycin Sulfate according to the labeled amount, and dissolve in 15 μL of Copper (II) sulfate TS and water to make 2 mL. Apply to Method 2: it meets the requirement.

Containers and storage Containers—Hermetic containers.

Perphenazine

Perphenazine, when dried, contains not less than 98.5% of C_{21}H_{26}ClN_{3}OS.

Description Perphenazine occurs as white to light yellow crystals or crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in methanol and in ethanol (95), soluble in acetic acid (100), sparingly soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

It is gradually colored by light.

Identification (1) Dissolve 5 mg of Perphenazine in 5 mL of sulfuric acid: a red color, changing to deep red-purple upon warming, is produced.

(2) Dissolve 0.2 g of Perphenazine in 2 mL of methanol, add this solution to 10 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25), and allow to stand for 4 hours. Collect the crystals, wash with a small volume of methanol, and dry at 105°C for 1 hour: the crystals so obtained melt between 237°C and 244°C (with decomposition).

(3) Determine the absorption spectrum of a solution of Perphenazine in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry 2.2.2, and compare the spectrum with the Reference Spectrum 1 or the spectrum of a solution of Perphenazine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the
same wavelengths. Separately, to 10 mL of the solution add 10 mL of water. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2 or the spectrum of a solution of Perphenazine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Perform the test with Perphenazine as directed under Flame Coloration Test <1.04> (2): a green color appears.

**Melting point** <2.60> 95 – 100°C

**Purity** (1) Heavy metals <1.07>—Proceed with 1.0 g of Perphenazine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Perform the test in the current of nitrogen in light-resistant containers under the protection from sunlight. Dissolve 0.10 g of Perphenazine in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add ethanol (95) to make exactly 10 mL. Pipet 1 mL of this solution, add ethanol (95) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.60>.

Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol and 1 mol/L ammonia TS (5:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the sample solution is not more intense than that from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 65°C, 4 hours).

**Residue on ignition** <2.46> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Perphenazine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-purple to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
\[ = 20.20 \text{ mg of C}_2\text{H}_2\text{ClN}_3\text{O}_S \]

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

**Perphenazine Tablets**

ペルフェナジン錠

Perphenazine Tablets contain not less than 90.0%, and not more than 110.0% of the labeled amount of perphenazine (C\(_2\)H\(_2\)ClN\(_3\)O\(_S\): 403.97).

**Method of preparation** Prepare as directed under Tablets, with Perphenazine.

**Identification** (1) Shake well a quantity of powdered Perphenazine Tablets, equivalent to 25 mg of Perphenazine according to the labeled amount, with 10 mL of methanol, and filter. Evaporate 2 mL of the filtrate on a water bath to dryness. With the residue, proceed as directed in the Identification (1) under Perphenazine.

(2) Add 5 mL of the filtrate obtained in the Identification (1) to 10 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25), and proceed as directed in the Identification (2) under Perphenazine.

(3) Determine the absorption spectrum of the filtrate obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 309 nm and 313 nm. Add 30 mL of methanol to another 10 mL of the filtrate, and determine the absorption spectrum: it exhibits a maximum between 256 nm and 260 nm.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Disintegrate 1 Perphenazine Tablet by shaking with 5 mL of water, shake well with 70 mL of methanol, and add methanol to make exactly 100 mL. Centrifuge this solution, pipet 1 mL of the supernatant liquid, add methanol to make exactly 50 mL of a solution containing about 4 μg of perphenazine (C\(_2\)H\(_2\)ClN\(_3\)O\(_S\)) in each mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Perphenazine RS, previously dried in vacuum over phosphorus (V) oxide at 65°C for 4 hours, dissolve in methanol to make exactly 250 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A\(_T\) and A\(_S\), of the sample solution and standard solution at 258 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of perphenazine (C\(_2\)H\(_2\)ClN\(_3\)O\(_S\))
\[ = M_S \times A_T/A_S \times V/V' \times 1/25 \]

M\(_S\): Amount (mg) of Perphenazine RS

**Dissolution** <6.10> When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 90 minutes of Perphenazine Tablets is not less than 70%.

Start the test with 1 tablet of Perphenazine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 10 mg of Perphenazine RS, previously dried in vacuum with phosphorus (V) oxide at 65°C for 4 hours, dissolve in 5 mL of 0.1 mol/L hydrochloric acid TS, and add the dissolution medium to make exactly 250 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A\(_T\) and A\(_S\), of the sample solution and standard solution at 258 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Perphenazine Tablets in 90 minutes is not less than 70%.

Dissolution rate (%) with respect to the labeled amount of perphenazine (C\(_2\)H\(_2\)ClN\(_3\)O\(_S\))
\[ = M_S \times A_T/A_S \times 1/C \times 36 \]

M\(_S\): Amount (mg) of Perphenazine RS
C: Labeled amount (mg) of perphenazine (C$_{21}$H$_{26}$ClN$_3$OS) in 1 tablet

**Assay** Weigh accurately and powder not less than 20 Perphenazine Tablets. Weigh accurately a portion of the powder, equivalent to about 4 mg of perphenazine (C$_{21}$H$_{26}$ClN$_3$OS), add 70 mL of methanol, shake well, and add methanol to make exactly 100 mL. Filter the solution, and discard the first 20 mL of the filtrate. Pipet 5 mL of the subsequent filtrate, add methanol to make exactly 50 mL, and use this solution as the sample solution. Weigh accurately about 10 mg of Perphenazine RS, previously dried in a vacuum over phosphorus (V) oxide at 65°C for 4 hours, and dissolve in methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A$_T$ and A$_S$, of the sample solution and the standard solution at 258 nm as directed under Ultraviolet-visible Spectrophotometry $<2.24>$.  

Amount (mg) of perphenazine (C$_{21}$H$_{26}$ClN$_3$OS)  
$= M_5 \times A_T/A_S \times 2/5$

M$_5$: Amount (mg) of Perphenazine RS

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

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**Perphenazine Maleate**  
ペルフェナジンマレイン酸塩

C$_{21}$H$_{26}$ClN$_3$OS.2C$_4$H$_4$O$_4$: 636.11  
2-[4-[3-(2-Chloro-10H-phenothiazin-10-yl)propyl]piperazin-1-yl]ethanol dimaleate  
[58-39-9, Perphenazine]

Perphenazine Maleate, when dried, contains not less than 98.0% of C$_{21}$H$_{26}$ClN$_3$OS.2C$_4$H$_4$O$_4$.

**Description** Perphenazine Maleate occurs as a white to light yellow powder. It is odorless.

It is sparingly soluble in acetic acid (100), slightly soluble in water and in ethanol (95), and practically insoluble in chloroform.

It dissolves in dilute hydrochloric acid.

It is gradually colored by light.

Melting point: about 175°C (with decomposition).

**Identification**  
(1) Dissolve 8 mg of Perphenazine Maleate in 5 mL of sulfuric acid: a red color is produced, which becomes deep red-purple on warming.

(2) Dissolve 0.3 g of Perphenazine Maleate in 3 mL of dilute hydrochloric acid, add 2 mL of water and 3 mL of ammonia solution (28), shake, and extract with three 10-mL portions of chloroform. [Reserve the aqueous layer, and use for test (5)]. Evaporate the combined chloroform extracts on a water bath to dryness, dissolve the residue in 20 mL of methanol, and pour into 10 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25). Allow to stand for 4 hours, collect the crystals, wash with a small amount of methanol, and dry at 105°C for 1 hour: the crystals melt $<2.60>^\circ$ between 237°C and 244°C (with decomposition).

(3) Determine the absorption spectrum of a solution of Perphenazine Maleate (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry $<2.24>$, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, to 10 mL of the solution add 30 mL of water. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry $<2.24>$, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Perform the test with Perphenazine Maleate as directed under Flame Coloration Test $<1.04>$ (2): a green color appears.

(5) Evaporate the aqueous layer reserved in (2) to dryness. To the residue add 1 mL of dilute sulfuric acid and 5 mL of water, and extract with four 25-mL portions of diethyl ether. Combine the diethyl ether extracts, and evaporate in a water bath at about 35°C with the aid of a current of air: the residue melts $<2.60>^\circ$ between 128°C and 136°C.

**Purity**  
(1) Heavy metals $<1.07>$—Proceed with 2.0 g of perphenazine maleate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic $<1.11>$—Prepare the test solution with 1.0 g of Perphenazine Maleate according to Method 3, and perform the test (not more than 2 ppm).

**Loss on drying** $<2.41>$ Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** $<2.44>$ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Perphenazine Maleate, previously dried, dissolve in 70 mL of acetic acid (100), and titrate $<2.59>$ with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
$= 31.81$ mg of C$_{21}$H$_{26}$ClN$_3$OS.2C$_4$H$_4$O$_4$

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

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**Perphenazine Maleate Tablets**  
ペルフェナジンマレイン酸塩錠

Perphenazine Maleate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of perphenazine maleate (C$_{21}$H$_{26}$ClN$_3$OS.2C$_4$H$_4$O$_4$: 636.11).

**Method of preparation** Prepare as directed under Tablets, with Perphenazine Maleate.
**Identification (1)** Shake a quantity of powdered Perphenazine Maleate Tablets, equivalent to 0.04 g of Perphenazine Maleate according to the labeled amount, with 5 mL of dilute hydrochloric acid and 30 mL of water, centrifuge, filter the supernatant liquid, add 3 mL of ammonia solution (28) to the filtrate, and extract with three 10-mL portions of chloroform. Reserve the aqueous layer, and use for test (4). Wash the combined chloroform extracts with two 5-mL portions of water, and separate the chloroform layer. Evaporate 6 mL of the chloroform solution on a water bath to dryness. Proceed with the residue as directed in the Identification (1) under Perphenazine Maleate.

(2) Evaporate 20 mL of the chloroform solution obtained in (1) on a water bath to dryness, dissolve the residue in 20 mL of methanol, and filter, if necessary. Warm the filtrate, add 5 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25), allow to stand for 4 hours, and proceed as directed in the Identification (2) under Perphenazine Maleate.

(3) To 2 mL of the filtrate obtained in the Assay add water to make 50 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24> it exhibits maxima between 253 nm and 257 nm and between 303 nm and 313 nm.

(4) Filter, if necessary, the aqueous layer reserved in (1), evaporate the filtrate to make about 5 mL, add 2 mL of dilute sulfuric acid, and extract with two 10-mL portions of diethyl ether. Combine the diethyl ether extracts, evaporate on a water bath to dryness, dissolve the residue in 5 mL of sulfuric acid TS, and add 1 to 2 drops of potassium permanganate TS: the red color of potassium permanganate TS fades immediately.

**Uniformity of dosage units**<6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Disintegrate 1 tablet of Perphenazine Maleate Tablets by shaking with 15 mL of 0.1 mol/L hydrochloric acid TS, shake vigorously with 30 mL of methanol, add water to make exactly 100 mL, and centrifuge. Pipet V mL of the supernatant liquid, add water to make exactly V mL of a solution containing about 6 μg of perphenazine maleate (C$_{21}$H$_{26}$ClN$_3$O$_5$.2C$_4$H$_4$O$_4$) in each mL, and use this solution as the sample solution. Separately, weigh accurately 30 mg of perphenazine maleate for assay, previously dried at 105°C for 3 hours, dissolve in 15 mL of 0.1 mol/L hydrochloric acid TS and 50 mL of methanol, add water to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, measure exactly 5 mL of the subsequent filtrate, add water to make exactly 250 mL, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of perphenazine maleate for assay, previously dried at 105°C for 3 hours, dissolve in 15 mL of 1 mol/L hydrochloric acid TS and 50 mL of methanol, add water to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, at 255 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

\[
\text{Dissolution rate (％) with respect to the labeled amount of perphenazine maleate (C$_{21}$H$_{26}$ClN$_3$O$_5$.2C$_4$H$_4$O$_4$) according to the labeled amount, and use this solution as the standard solution.}
\]

\[
M_S: \text{Amount (mg) of perphenazine maleate for assay}\]

\[
M_C: \text{Labeled amount (mg) of perphenazine maleate (C$_{21}$H$_{26}$ClN$_3$O$_5$.2C$_4$H$_4$O$_4$) in 1 tablet}\]

**Assay** Weigh accurately a portion of the powder, equivalent to about 40 mg of perphenazine maleate (C$_{21}$H$_{26}$ClN$_3$O$_5$.2C$_4$H$_4$O$_4$), shake well with 15 mL of 1 mol/L hydrochloric acid TS and 50 mL of methanol, add water to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, measure exactly 5 mL of the subsequent filtrate, add water to make exactly 250 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of perphenazine maleate for assay, previously dried at 105°C for 3 hours, dissolve in 15 mL of 1 mol/L hydrochloric acid TS and 50 mL of methanol, add water to make exactly 100 mL, Pipet 5 mL of this solution, add 3 mL of 0.1 mol/L hydrochloric acid TS, 10 mL of methanol and water to make exactly 250 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and the standard solution at 255 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

\[
\text{Amount (mg) of perphenazine maleate}\]

\[
M_S: \text{Amount (mg) of perphenazine maleate for assay}\]

\[
= M_S \times \frac{A_T}{A_S} \times \frac{V}{V} \times \frac{1}{C} \times \frac{45}{4}
\]

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.
Adsorbed Purified Pertussis Vaccine

Adsorbed Purified Pertussis Vaccine is a liquid for injection prepared by adding an aluminum salt to a liquid containing the protective antigen of Bordetella pertussis to make the antigen insoluble.

It conforms to the requirements of Adsorbed Purified Pertussis Vaccine in the Minimum Requirements for Biological Products.

Description Adsorbed Purified Pertussis Vaccine forms a homogeneous, white turbidity on shaking.

Pethidine Hydrochloride

Operidine

ペチジン塩酸塩

C_{15}H_{21}NO_2·HCl: 283.79
Ethyl 1-methyl-4-phenylpiperidine-4-carboxylate monohydrochloride [50-13-5]

Pethidine Hydrochloride, when dried, contains not less than 98.0% of C_{15}H_{21}NO_2·HCl.

Description Pethidine Hydrochloride occurs as a white, crystalline powder.

It is very soluble in water and in acetic acid (100), freely soluble in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution dissolved 1.0 g of Pethidine Hydrochloride in 20 mL of water is between 3.8 and 5.8.

Identification (1) Determine the absorption spectrum of a solution of Pethidine Hydrochloride (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pethidine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Pethidine Hydrochloride (1 in 50) responds to Qualitative Tests <1.699> (2) for chloride.

Melting point <2.68> 187 - 189°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Pethidine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 0.20 g of Pethidine Hydrochloride. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.240%).

(3) Related substances—Dissolve 0.05 g of Pethidine Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipe 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area obtained from both solutions by the automatic integration method: the total area of the peaks other than pethidine from the sample solution is not larger than the peak area of pethidine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 257 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.0 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (1 in 1000), adjust the pH to 3.0 with sodium hydroxide TS, and to 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pethidine is about 7 minutes.

Time span of measurement: About 2 times as long as the retention time of pethidine beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of pethidine obtained from 20 µL of this solution is equivalent to 5 to 15% of that from 20 µL of the standard solution.

System performance: To 2 mL each of the sample solution and a solution of isoamyl parahydroxybenzoate in the mobile phase (1 in 50,000) add the mobile phase to make 10 mL. When the procedure is run with 20 µL of this solution according to the above operating conditions, pethidine and isoamyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pethidine is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.5 g of Pethidine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.
Each mL of 0.1 mol/L perchloric acid VS  
= 28.38 mg of C_{15}H_{21}NO_{2}.HCl

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

### Pethidine Hydrochloride Injection

**Operidine Injection**

ペチジン塩酸塩注射液

Pethidine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of pethidine hydrochloride (C_{15}H_{21}NO_{2}.HCl: 283.79).

**Method of preparation** Prepare as directed under Injection, with Pethidine Hydrochloride.

**Description** Pethidine Hydrochloride Injection is a clear, colorless liquid.

It is affected by light.

pH 4.0 – 6.0

**Identification** Take a volume of Pethidine Hydrochloride Injection equivalent to 0.1 g of Pethidine Hydrochloride according to the labeled amount, and add water to make 200 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 250 nm and 254 nm, between 255 nm and 259 nm, and between 261 nm and 265 nm.

**Bacterial endotoxins** <4.01> Less than 6.0 EU/mg.

**Extractable volume** <6.05> It meets the requirement.

**Foreign insoluble matter** <6.06> Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Measure exactly a volume of Pethidine Hydrochloride Injection, equivalent to about 0.1 g of pethidine hydrochloride (C_{15}H_{21}NO_{2}.HCl), add exactly 10 mL of the internal standard solution, and add the mobile phase to make 50 mL. To 5 mL of this solution add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of pethidine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 10 mL of the internal standard solution, and add the mobile phase to make 50 mL. To 5 mL of this solution add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 20 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Qr and Qs, of the peak area of pethidine to that of the internal standard.

Amount (mg) of pethidine hydrochloride (C_{15}H_{21}NO_{2}.HCl)  
= M \times Qr/Qs

**Containers and storage** Containers—Tight containers.

### Hydrophilic Petrolatum

**Method of preparation**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Beeswax</td>
<td>80 g</td>
</tr>
<tr>
<td>Stearyl Alcohol or Cetanol</td>
<td>30 g</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>30 g</td>
</tr>
<tr>
<td>White Petrolatum</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 g

Melt and mix Stearyl Alcohol or Cetanol, White Beeswax and White Petrolatum on a water bath. Add Cholesterol, and melt completely by stirring. Stop warming, and stir until the mixture congeals.

**Description** Hydrophilic Petrolatum is white in color. It has a slight, characteristic odor. When mixed with an equal volume of water, it retains the consistency of ointment.

**Containers and storage** Containers—Tight containers.
White Petrolatum

White Petrolatum is a decolorized and purified mixture of hydrocarbons obtained from petroleum.

**Description** White Petrolatum is a white to pale yellow, homogeneous, unctuous mass. It is odorless and tasteless. It is practically insoluble in water, in ethanol (95) and in ethanol (99.5).

It dissolves in diethyl ether making a clear liquid or producing slight insoluble substances.

It becomes a clear liquid when warmed.

**Melting point** <2.60> 38 – 60°C (Method 3).

**Purity** (1) Color—Melt White Petrolatum by warming, and pour 5 mL of it into a test tube, and keep the content in a liquid condition: the liquid has no more color than the following control solution, when observed transversely from side against a white background.

Control solution: Add 3.4 mL of water to 1.6 mL of Iron (III) Chloride CS.

(2) Acidity or alkalinity—To 35.0 g of White Petrolatum add 100 mL of hot water, shake vigorously for 5 minutes, and then draw off the aqueous layer. Treat the White Petrolatum layer in the same manner using two 50-mL portions of hot water. To the combined aqueous layer add 1 drop of phenolphthalein TS, and boil: no red color is produced. Further add 2 drops of methyl orange TS: no red color is produced.

(3) Heavy metals <1.07>—Proceed with 1.0 g of White Petrolatum according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(4) Arsenic <1.1D>—Prepare the test solution with 1.0 g of White Petrolatum, according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 2 ppm).

(5) Sulfur compound—To 4.0 g of White Petrolatum add 2 mL of ethanol (99.5) and 2 drops of sodium hydroxide solution (1 in 5) saturated with lead (II) oxide, warm the mixture for 10 minutes at about 70°C with frequent shaking, and allow to cool: no dark color is produced.

(6) Organic acids—To 100 mL of dilute ethanol add 1 drop of phenolphthalein TS, and titrate with 0.01 mol/L sodium hydroxide VS, and 0.40 mL of 0.1 mol/L sodium hydroxide VS with vigorous shaking: the color of the solution remains red.

(7) Fats and fatty oils or resins—To 10.0 g of White Petrolatum add 50 mL of sodium hydroxide solution (1 in 5), and boil for 30 minutes under a reflux condenser. Cool the mixture, separate the aqueous layer, and filter, if necessary. To the aqueous layer add 200 mL of dilute sulfuric acid: neither oily matter nor precipitate is produced.

**Residue on ignition** <2.44> Not more than 0.05% (2 g).

**Containers and storage** Containers—Tight containers.

Yellow Petrolatum

Yellow Petrolatum is a purified mixture of hydrocarbons obtained from petroleum.

**Description** Yellow Petrolatum occurs as a yellow, homogeneous, unctuous mass. It is odorless and tasteless.

It is slightly soluble in ethanol (95), and practically insoluble in water.

It dissolves in diethyl ether, in petroleum benzine and in turpentine oil, making a clear liquid or producing slight insoluble substances.

It becomes a yellow, clear liquid with slight fluorescence when warmed.

**Melting point** <2.60> 38 – 60°C (Method 3).

**Purity** (1) Color—Melt Yellow Petrolatum by warming, and pour 5 mL of it into a test tube, and keep the content in a liquid condition: the liquid has no more color than the following control solution, when observed transversely from side against a white background.

Control solution: To 3.8 mL of Iron (III) Chloride CS add 1.2 mL of Cobalt (II) Chloride CS.

(2) Acidity or alkalinity—To 35.0 g of Yellow Petrolatum add 100 mL of hot water, shake vigorously for 5 minutes, and then draw off the aqueous layer. Treat the Yellow Petrolatum layer in the same manner using two 50-mL portions of hot water. To the combined aqueous layer add 1 drop of phenolphthalein TS, and boil: no red color is produced. Further add 2 drops of methyl orange TS: no red color is produced.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Yellow Petrolatum according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(4) Arsenic <1.1D>—Prepare the test solution with 1.0 g of Yellow Petrolatum, according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 2 ppm).

(5) Sulfur compound—To 4.0 g of Yellow Petrolatum add 2 mL of ethanol (99.5) and 2 drops of sodium hydroxide solution (1 in 5) saturated with lead (II) oxide, warm the mixture for 10 minutes at about 70°C with frequent shaking, and allow to cool: no dark color is produced.

(6) Organic acids—To 100 mL of dilute ethanol add 1 drop of phenolphthalein TS, and titrate with 0.01 mol/L sodium hydroxide VS, and 0.40 mL of 0.1 mol/L sodium hydroxide VS with vigorous shaking: the color of the solution remains red.

(7) Fats and fatty oils or resins—To 10.0 g of Yellow Petrolatum add 50 mL of sodium hydroxide solution (1 in 5), and boil for 30 minutes under a reflux condenser. Cool the mixture, separate the aqueous layer, and filter, if necessary. To the aqueous layer add 200 mL of dilute sulfuric acid: neither oily matter nor precipitate is produced.
**Phenethicillin Potassium**

フェネチシリンカリウム

\[
\text{C}_{17}\text{H}_{19}\text{KN}_{2}\text{O}_{5}\text{S}: 402.51
\]

Monopotassium \((2S,5R,6R)-3,3\text{-dimethyl-7-oxo-6-\[2(2RS)-2\text{-phenoxypropanoylaminol-4-thia-1-azabicyclo[3.2.0]hept-2-carboxylate[132-93-4]}}

Phenethicillin Potassium contains not less than 1400 units and not more than 1480 units per mg, calculated on the dried basis. The potency of Phenethicillin Potassium is expressed as unit based on the amount of phenethicillin potassium \((\text{C}_{17}\text{H}_{19}\text{KN}_{2}\text{O}_{5}\text{S})\). One unit of Phenethicillin Potassium is equivalent to 0.68 \(\mu\)g of phenethicillin potassium \((\text{C}_{17}\text{H}_{19}\text{KN}_{2}\text{O}_{5}\text{S})\).

**Description** Phenethicillin Potassium occurs as a white to light yellowish white crystalline powder.

It is freely soluble in water, and slightly soluble in ethanol (99.5).

**Identification**

(1) Determine the absorption spectrum of a solution of Phenethicillin Potassium \((1 \text{ in 5000})\) as directed under Ultraviolet-visible Spectrophotometry \(<2.24\rangle\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Phenethicillin Potassium as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25\rangle\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Phenethicillin Potassium responds to Qualitative Tests \(<1.09\rangle\) (1) for potassium salt.

**Optical rotation** \(<2.49\rangle\) \([\alpha]_{D}^{20}+217 \text{ to } +244^\circ\) (1 g calculated on the dried basis, phosphate TS, 100 mL, 100 mm).

\(L\)-\(\alpha\)-Phenethicillin potassium Dissolve about 50 mg of Phenethicillin Potassium in the mobile phase to make 50 mL, and use this solution as the sample solution. Perform the test with 10 \(\mu\)L of the sample solution as directed under Liquid Chromatography \(<2.02\rangle\) according to the following conditions, and determine the peak areas, \(A_{0}\) and \(A_{L}\), of \(\alpha\)-phenethicillin and \(L\)-\(\alpha\)-phenethicillin by the automatic integration method: \(A_{L}/(A_{0} + A_{L})\) is between 0.50 and 0.70.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wave-length: 254 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \(\mu\)m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Adjust the pH of a mixture of a solution of
Phenobarbital / Official Monographs

Phenobarbital is a white crystalline powder. It is very soluble in N,N-dimethylformamide, freely soluble in ethanol (95) and in acetone, sparingly soluble in acetonitrile, and very slightly soluble in water. It dissolves in sodium hydroxide TS. The pH of a saturated solution of Phenobarbital is between 5.0 and 6.0.

Identification (1) Determine the absorption spectrum of a solution of Phenobarbital in boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Phenobarbital as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.6b> 175 – 179°C

Phenobarbital, when dried, contains not less than 99.0% and not more than 101.0% of C₁₂H₁₅N₂O₃.

Description Phenobarbital occurs as white crystals or crystalline powder.

For the standard solution, proceed as directed in the operating conditions for the L-α-phenethicillin potassium. Time span of measurement: About 1.5 times as long as the retention time of L-α-phenethicillin.

System suitability—
Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine each peak area by the automatic integration method: the total of the peak areas of 5-Ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione [50-06-6]

C₁₂H₁₂N₂O₃: 232.24
5-Ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione

for exactly 15 minutes. Add 0.2 – 0.5 mL of dilute nitric acid and titrate with 0.01 mol/L sodium thiosulfate VS until the color of the solution disappears. Separately, to exactly 2 mL each of the sample solution and standard solution add exactly 10 mL of 0.005 mol/L iodine VS, then proceed in the same manner as above allowing to stand for 15 minutes as a blank determination, and make any necessary correction. Determine the volumes, V₁ and V₃, of 0.005 mol/L iodine VS consumed in the sample solution and standard solution.

Amount (unit) of C₁₂H₁₅N₂O₃ = Mₛ × V₁/V₃

Mₛ: Amount (unit) of Phenethicillin Potassium RS

Containers and storage Containers—Well-closed containers.

Dissolve 50 mg of Phenethicillin Potassium according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.17>—Prepare the test solution with 1.0 g of Phenethicillin Potassium according to Method 4 and, perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Phenethicillin Potassium in 50 mL of the mobile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine each peak area by the automatic integration method: the total of the peak areas other than D-α-phenethicillin and L-α-phenethicillin obtained from the sample solution is not larger than 5 times the total of the peak areas of D-α-phenethicillin and L-α-phenethicillin from the standard solution.

Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions for the L-α-Phenethicillin potassium.

Time span of measurement: About 1.5 times as long as the retention time of L-α-phenethicillin.

System suitability—
Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of L-α-phenethicillin obtained from 10 μL of this solution is about 25 minutes.

The pH of a saturated solution of Phenobarbital is between 5.0 and 6.0.

Phenobarbital, when dried, contains not less than 99.0% and not more than 101.0% of C₁₂H₁₅N₂O₃.

Description Phenobarbital occurs as white crystals or crystalline powder.

Identification (1) Determine the absorption spectrum of a solution of Phenobarbital in boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Phenobarbital as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.6b> 175 – 179°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Phenobarbital in 5 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) Chloride <1.07>—Dissolve 0.30 g of Phenobarbital in 20 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as

Phenobarbital

C₁₂H₁₂N₂O₃: 232.24
5-Ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione

[50-06-6]
the test solution. Prepare the control solution as follows: take 0.30 mL of 0.01 mol/L hydrochloric acid VS, 20 mL of acetone and 6 mL of dilute nitric acid, and add water to make 50 mL (not more than 0.035%).

(3) Heavy metals <1.07—Proceed with 1.0 g of Phenobarbital according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead solution (not more than 20 ppm).

(4) Phenylbarbituric acid—Boil 1.0 g of Phenobarbital with 5 mL of ethanol (95) for 3 minutes: the solution is clear.

(5) Related substances—Dissolve 0.10 g of Phenobarbital in 100 mL of acetonitrile, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the area of the peak other than phenobarbital obtained from the sample solution is not larger than the peak area of phenobarbital from the standard solution.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of water and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of phenobarbital is about 5 minutes.

Time span of measurement: About 12 times as long as the retention time of phenobarbital, beginning after the solvent peak.

**System suitability—**

Test for required detectability: Pipet 5 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of phenobarbital obtained with 10 µL of this solution is equivalent to 20 to 30% of that with 10 µL of the standard solution.

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of phenobarbital are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of phenobarbital is not more than 3.0%.

**Loss on drying <2.41>** Not more than 1.0% (1 g, 105°C, 2 hours).

**Residue on ignition <2.44>** Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Phenobarbital, previously dried, dissolve in 50 mL of N,N-dimethylformamide, and titrate <2.59> with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution change from yellow to yellow-green (indicator: 1 mL of alizarin yellow G-G-thymolphthalein TS). Perform a blank determination using a mixture of 50 mL of N,N-dimethylformamide and 22 mL of ethanol (95), and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 23.22 mg of C_{12}H_{12}N_{2}O_{3}.

**Containers and storage** Containers—Well-closed containers.

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**10% Phenobarbital Powder**

### Phenobarbital Powder

フェノバルビタール散 10%

10% Phenobarbital Powder contains not less than 9.3% and not more than 10.7% of phenobarbital (C_{12}H_{12}N_{2}O_{3} : 232.24).

#### Method of preparation

<table>
<thead>
<tr>
<th>Phenobarbital</th>
<th>100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch, Lactose Hydrate or their mixture</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

To make 1000 g

Prepare as directed under Granules or Powders, with the above ingredients.

**Identification** (1) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 238 nm and 242 nm.

(2) To 6 g of 10% Phenobarbital Powder add 150 mL of ethanol, shake well, and filter. Condense the filtrate on a water bath to about 5 mL, add about 50 mL of water, filter to collect the formed crystals, and dry them at 105°C for 2 hours. Determine the infrared absorption spectrum of the crystals as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Dissolution <6.10>** When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of 10% Phenobarbital Powder is not less than 80%.

Start the test with an accurately weighed about 0.3 g of 10% Phenobarbital Powder, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 µm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 10 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6, and use this solution as the sample solution. Separately, weigh accurately about 17 mg of phenobarbital for assay, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 10 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under...
Ultraviolet-visible Spectrophotometry <2.24>, using a mixture of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6, and water (2:1) as the blank, and determine the absorbances, \( A_1 \) and \( A_2 \), at 240 nm.

Dissolution rate (%) with respect to the labeled amount of phenobarbital (C\(_{12}\)H\(_{12}\)N\(_2\)O\(_3\))

\[
M_5 = \frac{M_T}{A_1/A_2} \times \frac{1}{C} \times 180
\]

\( M_5 \): Amount (mg) of phenobarbital for assay

\( M_T \): Amount (g) of 10% Phenobarbital Powder

\( C \): Labeled amount (mg) of phenobarbital (C\(_{12}\)H\(_{12}\)N\(_2\)O\(_3\)) in 1 g

Assay

Weigh accurately about 0.2 g of 10% Phenobarbital Powder, dissolve in a boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 to make exactly 100 mL. Pipet 5 mL of this solution, add a boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of phenobarbital for assay, previously dried at 105°C for 2 hours, and add a boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 to make exactly 100 mL. Pipet 5 mL of this solution, add a boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 as the blank, and determine the absorbances, \( A_1 \) and \( A_2 \), at 240 nm.

\[
\text{Amount (mg) of phenobarbital (C}_{12}\text{H}_{12}\text{N}_{2}\text{O}_{3}) = M_5 \times \frac{A_1}{A_2} \times \frac{1}{C} \times 180
\]

\( M_5 \): Amount (mg) of phenobarbital for assay

Containers and storage

Containers—Well-closed containers.

Phenol

Carbolic Acid

フェノール

\[
\text{C}_6\text{H}_6\text{O}: 94.11
\]

Phenol for Disinfection contains not less than 98.0% of \( \text{C}_6\text{H}_6\text{O} \).

Description

Phenol occurs as colorless to slightly red crystals, crystalline masses, or liquid containing these crystals. It has a characteristic odor. It is very soluble in ethanol (95) and in diethyl ether, and freely soluble in water.

Phenol (10 g) is liquefied by addition of 1 mL of water. The color changes gradually through red to dark red by light or air.

It cauterizes the skin, turning it white.

Congealing point: about 40°C

Identification

(1) Add 1 drop of iron (III) chloride TS to 10 mL of a solution of Phenol (1 in 100): a blue-purple color develops.

(2) Add bromine TS dropwise to 5 mL of a solution of Phenol (1 in 10,000): a white precipitate is produced, which at first dissolves with shaking, but becomes permanent as excess of the reagent is added.

Purity

(1) Clarity and color of solution and acidity or alkalinity—Dissolve 1.0 g of Phenol in 15 mL of water: the solution is clear, and neutral or only faintly acid. Add 2 drops of methyl orange TS: no red color develops.

(2) Residue on evaporation—Weigh accurately about 5 g of Phenol, evaporate on a water bath, and dry the residue at 105°C for 1 hour: the mass is not more than 0.05% of the mass of the sample.

Assay

Dissolve about 1.5 g of Phenol, accurately weighed, in water to make exactly 1000 mL. Transfer exactly 25 mL of this solution to an iodine flask, add exactly 30 mL of 0.05 mol/L bromine VS, then 5 mL of hydrochloric acid, and immediately stopper the flask. Shake the flask repeatedly for 30 minutes, allow to stand for 15 minutes, then add 7 mL of potassium iodide TS, at once stopper the flask, and shake well. Add 1 mL of chloroform, stop the flask, and shake thoroughly. Titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS = 1.569 mg of \( \text{C}_6\text{H}_6\text{O} \)

Containers and storage

Containers—Tight containers.

Storage—Light-resistant.

Phenol for Disinfection

Carbolic Acid for Disinfection

消毒用フェノール

Phenol for Disinfection contains not less than 95.0% of phenol (\( \text{C}_6\text{H}_6\text{O} \): 94.11).

Description

Phenol for Disinfection occurs as colorless to slightly red crystals, crystalline masses, or liquid containing these crystals. It has a characteristic odor.

Phenol for Disinfection (10 g) is liquefied by addition of 1 mL of water.

It cauterizes the skin, turning it white.

Congealing point: about 30°C

Identification

(1) To 10 mL of a solution of Phenol for Disinfection (1 in 100) add 1 drop of iron (III) chloride TS: a blue-purple color is produced.

(2) To 5 mL of a solution of Phenol for Disinfection (1 in 10,000) add bromine TS dropwise: a white precipitate is formed, and it dissolves at first upon shaking but becomes permanent as excess of the reagent is added.

Purity

(1) Clarity of solution—Dissolve 1.0 g of Phenol for Disinfection in 15 mL of water: the solution is clear.

(2) Residue on evaporation—Weigh accurately about 5 g
of Phenol for Disinfection, evaporate on a water bath, and
dry the residue at 105°C for 1 hour: the mass is not more
than 0.10% of the mass of the sample.

**Assay** Dissolve about 1 g of Phenol for Disinfection, accu-
rately weighed, in water to make exactly 1000 mL. Pipet 25
mL of the solution into an iodine flask, add exactly 30 mL of
0.05 mol/L bromine VS and 5 mL of hydrochloric acid,
stopper immediately, shake for 30 minutes and allow to
stand for 15 minutes. Add 7 mL of potassium iodide TS,
stopper immediately, shake well, and titrate <2.50> the liber-
ated iodine with 0.1 mol/L sodium thiosulfate VS (indicator:
1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of C₆H₆O

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

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**Liquefied Phenol**

**Liquefied Carbolic Acid**

液状フェノール

Liquefied Phenol is Phenol maintained in a liquid
condition by the presence of 10% of Water, Purified
Water or Purified Water in Containers.

It contains not less than 88.0% of phenol (C₆H₆O: 94.11)

**Description** Liquefied Phenol is a colorless or slightly red-
dish liquid. It has a characteristic odor.

It is miscible with ethanol (95), with diethyl ether and with
glycerin.

A mixture of equal volumes of Liquefied Phenol and glycer-
in is miscible with water.

The color changes gradually to dark red on exposure to
light or air.

It cauterizes the skin, turning it white.

Specific gravity d₂₀: about 1.065

**Identification (1)** Add 1 drop of iron (III) chloride TS to
10 mL of a solution of Liquefied Phenol (1 in 100): a blue-
purple color develops.

(2) Add bromine TS dropwise to 5 mL of a solution of
Liquefied Phenol (1 in 10,000): a white precipitate is pro-
duced, which at first dissolves with shaking, but becomes
permanent as excess of the reagent is added.

**Boiling point** <2.57> Not more than 182°C.

**Purity** (1) Clarity and color of solution and acidity or
alkalinity—Dissolve 1.0 g of Liquefied Phenol in 15 mL of
water: the solution is clear, and neutral or only faintly acid.
Add 2 drops of methyl orange TS: no red color develops.

(2) Residue on evaporation—Weigh accurately about 5 g
of Liquefied Phenol, evaporate on a water bath, and dry the
residue at 105°C for 1 hour: the mass is not more than
0.05% of the mass of the sample.

**Assay** Dissolve about 1.7 g of Liquefied Phenol, accurately
weighed, in water to make exactly 1000 mL. Transfer ex-
actly 25 mL of this solution to an iodine flask, add exactly 30
mL of 0.05 mol/L bromine VS, then 5 mL of hydrochloric
acid, and immediately stopper the flask. Shake the flask
repeatedly for 30 minutes, allow to stand for 15 minutes,
then add 7 mL of potassium iodide TS, at one stopper the
flask tightly, and shake well. Add 1 mL of chloroform, stop-
per the flask, and shake thoroughly. Titrate <2.50> the liber-
ated iodine with 0.1 mol/L sodium thiosulfate VS (indicator:
1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of C₆H₆O

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

---

**Dental Phenol with Camphor**

歯科用フェノール・カンフル

**Method of preparation**

<table>
<thead>
<tr>
<th></th>
<th>Phenol</th>
<th>d- or dl-Camphor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35 g</td>
<td>65 g</td>
</tr>
</tbody>
</table>

Melt Phenol by warming, add d-Camphor or dl- Cam-
phor, and mix.

**Description** Dental Phenol with Camphor is a colorless or
light red liquid. It has a characteristic odor.

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

---

**Phenol and Zinc Oxide Liniment**

フェノール・亜鉛華リニメント

**Method of preparation**

<table>
<thead>
<tr>
<th></th>
<th>Liquefied Phenol</th>
<th>Powdered Tragacanth</th>
<th>Carmellose Sodium</th>
<th>Glycerin</th>
<th>Zinc Oxide</th>
<th>Purified Water or Purified Water in Containers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22 mL</td>
<td>20 g</td>
<td>30 g</td>
<td>30 mL</td>
<td>100 g</td>
<td>a sufficient quantity</td>
</tr>
</tbody>
</table>

Mix Liquefied Phenol, Glycerin and Purified Water or
Purified Water in Containers, add Powdered Tragacanth in
small portions by stirring, and allow the mixture to stand
overnight. To the mixture add Carmellose Sodium in small
portions by stirring to make a pasty mass, add Zinc Oxide in
small portions, and mix. Less than 5 g of Powdered
Tragacanth or Carmellose Sodium can be replaced by each
other to make 50 g in total.

**Description** Phenol and Zinc Oxide Liniment is a white,
pasty mass. It has a slight odor of phenol.

**Identification (1)** Shake well 1 g of Phenol and Zinc
Oxide Liniment with 10 mL of diethyl ether, and filter. To
the filtrate add 10 mL of dilute sodium hydroxide TS, shake
well, and separate the water layer. To 1 mL of the water layer add 1 mL of sodium nitrite TS and 1 mL of dilute hydrochloric acid, shake, and add 3 mL of sodium hydroxide TS: a yellow color develops (phenol).

(2) Place 1 g of Phenol and Zinc Oxide Liniment in a porcelain crucible, heat gradually raising the temperature until the content is charred, and then ignite it strongly: a yellow color develops, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. To the filtrate add 2 to 3 drops of potassium hexacyanoferrate(II) TS: a white precipitate is produced (zinc oxide).

(3) Shake 0.5 g of Phenol and Zinc Oxide Liniment with 1 mL of water and 5 mL of chloroform, separate the chloroform layer, and use this solution as the sample solution. Separately, dissolve 0.01 g of phenol in 5 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography.<2.03>. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia solution (28) (50:5:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spots obtained from the sample solution and the standard solution show the same Rf value.

Containers and storage
Containers—Tight containers.

Phenolated Water

フェノール水

Phenolated Water contains not less than 1.8 w/v% and not more than 2.3 w/v% of phenol (C₆H₆O: 94.11).

Method of preparation

Liquefied Phenol 22 mL
Water, Purified Water or Purified Water in Containers a sufficient quantity

To make 1000 mL

Mix the above ingredients.

Description Phenolated Water is a colorless, clear liquid, having the odor of phenol.

Identification (1) Add 1 drop of iron (III) chloride TS to 10 mL of Phenolated Water: a blue-purple color develops.

(2) To 5 mL of a solution of Phenolated Water (1 in 200) add bromine TS dropwise: a white precipitate is formed, and it dissolves at first upon shaking but becomes permanent as excess of the reagent is added.

Assay Take exactly 2 mL of Phenolated Water into an iodine flask, add 25 mL of water, then add exactly 40 mL of 0.05 mol/L bromine VS and 5 mL of hydrochloric acid, stopper immediately, shake for 30 minutes, and allow to stand for 15 minutes. Add 7 mL of potassium iodide TS, stopper tightly at once, shake well, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS = 1.569 mg of C₆H₆O

Containers and storage
Containers—Tight containers.

Phenolated Water for Disinfection

消毒用フェノール水

Phenolated Water for Disinfection contains not less than 2.8 w/v% and not more than 3.3 w/v% of phenol (C₆H₆O: 94.11).

Method of preparation

Phenol for Disinfection 31 g
Water, Purified Water or Purified Water in Containers a sufficient quantity

To make 1000 mL

Mix the above ingredients.

Description Phenolated Water for Disinfection is a clear, colorless liquid, having the odor of phenol.

Identification (1) Add 1 drop of iron (III) chloride TS to 10 mL of Phenolated Water for Disinfection: a blue-purple color develops.

(2) Proceed with 5 mL of a solution of Phenolated Water for Disinfection (1 in 200) as directed in the Identification (2) under Phenol for Disinfection.

Assay Take exactly 5 mL of Phenolated Water for Disinfection, add water to make exactly 100 mL, then pipet 25 mL of the solution into an iodine flask, and proceed as directed in the Assay under Phenol for Disinfection.

Each mL of 0.05 mol/L bromine VS = 1.569 mg of C₆H₆O

Containers and storage
Containers—Tight containers.

Phenolsulfonphthalein

フェノールスルホンフタレイン

Phenolsulfonphthalein, when dried, contains not less than 98.0% of C₁₉H₁₄O₅S.

Description Phenolsulfonphthalein occurs as a vivid red to dark red, crystalline powder.

It is very slightly soluble in water and in ethanol (95).

It dissolves in sodium hydroxide TS.

Identification (1) Dissolve 5 mg of Phenolsulfonphthalein in 5 mL of dilute hydrochloric acid, and add 1 mL of sodium nitrite TS and 3 mL of sodium hydroxide TS: a yellow color develops (phenol).
Phenolsulfonphthalein Injection

フェノールスルホンフタレイン注射液

Phenolsulfonphthalein Injection is an aqueous solution for injection.

It contains not less than 0.54 w/v% and not more than 0.63 w/v% of phenolsulfonphthalein (C_{19}H_{14}O_{5}S: 354.38).

Method of preparation

| Phenolsulfonphthalein | 6 g |
| Sodium Chloride       | 9 g |
| Sodium Bicarbonate    | 1.43 g |
| (or Sodium Hydroxide) | 0.68 g |
| Water for Injection or Sterile Water | a sufficient quantity |

Prepare as directed under Injections, with the above ingredients.

Description Phenolsulfonphthalein Injection is a clear, orange-yellow to red liquid.

Identification To 1 mL of Phenolsulfonphthalein Injection add 2 to 3 drops of sodium hydroxide TS, and proceed as directed in the Identification (1) under Phenolsulfonphthalein.

pH 2.54–6.0–7.6

Bacterial endotoxins 4.01 Less than 7.5 EU/mg.

Extractable volume 6.05 It meets the requirement.

Foreign insoluble matter 6.06 Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter 6.07 Perform the test according to Method 2: it meets the requirement.

Sterility 4.06 Perform the test according to the Membrane filtration method: it meets the requirement.

Sensitivity To 1.0 mL of Phenolsulfonphthalein Injection add 5 mL of water. To 0.20 mL of this solution add 50 mL of freshly boiled and cooled water and 0.40 mL of 0.01 mol/L sodium hydroxide VS: a deep red-purple color develops, and it changes to light yellow on the addition of 0.40 mL of 0.005 mol/L sulfuric acid VS.

Assay Pipet 5 mL of Phenolsulfonphthalein Injection, and add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 250 mL. Pipet 5 mL of this solution, add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of phenolsulfonphthalein for assay, previously dried in a desiccator (sili- ca gel) for 4 hours, and dissolve in a solution of anhydrous sodium carbonate (1 in 100) to make exactly 250 mL. Pipet 5 mL of this solution, add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 200 mL, and use this solution as the sample solution. Determine the absorbances, A_f and A_s, of the sample solution and standard solution at 559 nm as directed under Ultraviolet-visible Spectropho- tometry 2.24.
1-Phenylalanine / Official Monographs

Amount (mg) of phenolsulfonphthalein (C_{19}H_{12}O_{2}S) = M_{S} \times A_{T}/A_{S}

M_{S}: Amount (mg) of phenolsulfonphthalein for assay

Containers and storage Containers—Hermetic containers.

1-Phenylalanine

L-フェニルアラニン

C_{9}H_{11}NO_{2}: 165.19
(2S)-2-Amino-3-phenylpropanoic acid

L-Phenylalanine, when dried, contains not less than 98.5% of C_{9}H_{11}NO_{2}.

Description L-Phenylalanine occurs as white crystals or crystalline powder. It is odorless or has a faint characteristic odor, and has a slightly bitter taste.

It is freely soluble in formic acid, sparingly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification Determine the infrared absorption spectrum of L-Phenylalanine, previously dried, as directed in the procedure for KBr disks.

Optical rotation <2.40° [α]_{D}^{20}: -33.0° to -35.5° (after drying, 0.5 g, water, 25 mL, 100 mm).

pH <2.54 Dissolve 0.2 g of L-Phenylalanine in 20 mL of water: the pH of this solution is between 5.3 and 6.3.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of L-Phenylalanine in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride <1.0 ppm—Perform the test with 0.5 g of L-Phenylalanine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14—Perform the test with 0.6 g of L-Phenylalanine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02—Perform the test with 0.25 g of L-Phenylalanine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07—Dissolve 1.0 g of L-Phenylalanine in 40 mL of water and 2 mL of dilute acetic acid by warming, cool, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(6) Arsenic <1.10—Dissolve 1.0 g of L-Phenylalanine in 5 mL of dilute hydrochloric acid and 15 mL of water, and perform the test with this solution as the test solution (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of L-Phenylalanine in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, and add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.17 g of L-Phenylalanine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 16.52 mg of C_{9}H_{11}NO_{2}

Containers and storage Containers—Tight containers.

Phenylbutazone

フェニルプタゾン

C_{19}H_{20}N_{2}O_{2}: 308.37
4-Butyl-1,2-diphenylpyrazolidine-3,5-dione

Phenylbutazone, when dried, contains not less than 99.0% of C_{19}H_{20}N_{2}O_{2}.

Description Phenylbutazone occurs as a white to slightly yellowish white, crystalline powder. It is odorless, and is at first tasteless but leaves a slightly bitter aftertaste.

It is freely soluble in acetone, soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Identification (1) To 0.1 g of Phenylbutazone add 1 mL of acetic acid (100) and 1 mL of hydrochloric acid, and heat on a water bath under a reflux condenser for 30 minutes. Add 10 mL of water, and cool with ice water. Filter, and to the filtrate add 3 to 4 drops of sodium nitrite TS. To 1 mL of this solution add 1 mL of 2-naphthol TS and 3 mL of chloroform, and shake: a deep red color develops in the chloroform layer.
(2) Dissolve 1 mg of Phenylbutazone in 10 mL of dilute sodium hydroxide solution, and dilute with water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <2.60> 104 – 107°C

Purity (1) Clarity of solution—Dissolve 1.0 g of Phenylbutazone in 20 mL of sodium hydroxide solution (2 in 25), and allow to stand at 25 ± 1°C for 3 hours: the solution is clear. Determine the absorbance of this solution at 420 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: it is not more than 0.05.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Phenylbutazone according to Method 2, and prepare the test solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.1D>—Prepare the test solution with 1.0 g of Phenylbutazone, according to Method 3, and perform the test (not more than 2 ppm).

(4) Readily carbonizable substances—Dissolve 1.0 g of Phenylbutazone in 20 mL of sulfuric acid, and allow to stand at 25 ± 1°C for exactly 30 minutes: the solution is clear. Determine the absorbance of this solution at 420 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: it is not more than 0.10.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Phenylbutazone, previously dried, dissolve in 25 mL of acetone, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the solution shows a blue color which persists for 15 seconds (indicator: 5 drops of bromothymol blue TS). Perform a blank determination with a mixture of 25 mL of acetone and 16 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 30.84 mg of C19H20N2O2

Containers and storage Containers—Tight containers.

Phenylephrine Hydrochloride

フェニレフリン塩酸塩

C9H13NO2.HCl: 203.67
(1R)-1-(3-Hydroxyphenyl)-2-methylaminoethanol monohydrochloride [61-76-7]

Phenylephrine Hydrochloride, when dried, contains not less than 98.0% and not more than 102.0% of C9H13NO2.HCl.

Description Phenylephrine Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in water, freely soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Phenylephrine Hydrochloride (1 in 100) is between 4.5 and 5.5.

Identification (1) To 1 mL of a solution of Phenylephrine Hydrochloride (1 in 100) add 1 drop of copper (II) sulfate TS and 1 mL of a solution of sodium hydroxide (1 in 5): a blue color is produced. To the solution so obtained add 1 mL of diethyl ether, and shake vigorously: no blue color develops in the diethyl ether layer.

(2) To 1 mL of a solution of Phenylephrine Hydrochloride (1 in 100) add 1 drop of iron (III) chloride TS: a persistent purple color is produced.

(3) Dissolve 0.3 g of Phenylephrine Hydrochloride in 3 mL of water, add 1 mL of ammonia TS, and rub the inner side of the test tube with a glass rod: a precipitate is produced. Collect the precipitate, wash with a few drops of ice-cold water, and dry at 105°C for 2 hours: it melts <2.60> between 170°C and 177°C.

(4) A solution of Phenylephrine Hydrochloride (1 in 100) responds to Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49> [α]D: −42.0 – −47.5° (after drying, 0.5 g, water, 10 mL, 100 mm).

Melting point <2.60> 140 – 145°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Phenylephrine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <1.1D>—Take 0.5 g of Phenylephrine Hydrochloride, and perform the test. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Ketone—Dissolve 0.20 g of Phenylephrine Hydrochloride in 1 mL of water, and add 2 drops of sodium pentacyanonirotosylferrate (III) TS, 1 mL of sodium hydroxide TS and then 0.6 mL of acetic acid (100): the solution has no more color than the following control solution.

Control solution: Prepare as directed above without Phenylephrine Hydrochloride.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.1 g of Phenylephrine Hydrochloride, previously dried, dissolve in 40 mL of water contained in an iodine flask, add exactly measured 50 mL of 0.05 mol/L bromine VS, then add 5 mL of hydrochloric acid, and immediately stopper tightly. Shake the mixture, and allow to stand for 15 minutes. To this solution add 10 mL of potassium iodide TS carefully, stopper tightly immediately, shake thoroughly, allow to stand for 5 minutes, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS = 3.395 mg of C19H20N2O2

Containers and storage Containers—Light-resistant.

Storage—Light-resistant.
Phenytoin

Diphenylhydantoin

フェニトイン

C₁₅H₁₂N₂O₂: 252.27
5,5-Diphenylimidazolidine-2,4-dione [37-41-0]

Phenytoin, when dried, contains not less than 99.0% of C₁₅H₁₂N₂O₂.

**Description** Phenytoin occurs as a white, crystalline powder or granules. It is odorless and tasteless.

It is sparingly soluble in ethanol (95) and in acetone, slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Melting point: about 296°C (with decomposition).

**Identification**

(1) Dissolve 0.02 g of Phenytoin in 2 mL of ammonia TS, and add 5 mL of silver nitrate TS: a white precipitate is produced.

(2) Boil a mixture of 0.01 g of Phenytoin, 1 mL of ammonia TS and 1 mL of water, and add dropwise 2 mL of a mixture prepared from 50 mL of a solution of copper (II) sulfate pentahydrate (1 in 20) and 10 mL of ammonia TS: a red, crystalline precipitate is produced.

(3) Heat 0.1 g of Phenytoin with 0.2 g of sodium hydroxide, and fuse: the gas evolved turns moistened red litmus paper blue.

(4) Add 3 mL of chlorinated lime TS to 0.1 g of Phenytoin, shake for 5 minutes, and dissolve the oily precipitate in 15 mL of hot water. After cooling, add 1 mL of dilute hydrochloric acid dropwise, then add 4 mL of water. Filter the white precipitate thus obtained, wash with water, and press it with dry filter paper to remove the accompanying water. Dissolve the precipitate with 1 mL of chloroform, add 5 mL of dilute ethanol (9 in 10), and rub the inner surface of the flask to produce a white, crystalline precipitate. Collect the precipitate, wash with ethanol (95), and dry: the melting point <2.60° is between 165°C and 169°C.

**Purity**

(1) Clarity and color of solution—Dissolve 0.20 g of Phenytoin in 10 mL of 0.2 mol/L sodium hydroxide VS: the solution is clear and colorless. Then heat the solution: no turbidity is produced. Cool, and mix the solution with 5 mL of acetone: the solution is clear and colorless.

(2) Acidity or alkalinity—Shake 2.0 g of Phenytoin with 40 mL of water for 1 minute, filter, and perform the following tests using this filtrate as the sample solution.

(i) To 10 mL of the sample solution add 2 drops of phenolphthalein TS: no color develops. Then add 0.15 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(ii) To 10 mL of the sample solution add 0.30 mL of 0.01 mol/L hydrochloric acid VS and 5 drops of methyl red TS: a red to orange color develops.

(3) Chloride <1.0%—Dissolve 0.30 g of Phenytoin in 30 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution from 0.60 mL of 0.01 mol/L hydrochloric acid VS, 30 mL of acetone and 6 mL of dilute nitric acid, and add water to 50 mL (not more than 0.071%).

(4) Heavy metals <1.0%—Proceed with 1.0 g of Phenytoin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** <2.4% Not more than 0.5% (2 g, 105°C, 2 hours).

**Residue on ignition** <2.4% Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Phenytoin, previously dried, dissolve in 40 mL of ethanol (95) with the aid of gentle heating, add 0.5 mL of thymolphthalein TS immediately, and titrate with 0.1 mol/L sodium hydroxide VS until a light blue color develops. Then add 1 mL of pyridine, 5 drops of phenolphthalein TS and 25 mL of silver nitrate TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until a light red color, which persists for 1 minute, develops.

Each mL of 0.1 mol/L sodium hydroxide VS = 25.23 mg of C₁₅H₁₂N₂O₂

**Containers and storage** Containers—Well-closed containers.

Phenytoin Powder

Diphenylhydantoin Powder

フェニトイン散

Phenytoin Powder contains not less than 95.0% and not more than 105.0% of the labeled amount of phenytoin (C₁₅H₁₂N₂O₂: 252.27).

**Method of preparation** Prepare as directed under Granules or Powders, with Phenytoin.

**Identification** Weigh a portion of Phenytoin Powder, equivalent to 0.3 g of Phenytoin according to the labeled amount, stir well with two 100-mL portions of diethyl ether, and extract. Combine the diethyl ether extracts, and filter. Evaporate the filtrate on a water bath to dryness, and proceed with the residue as directed in the Identification under Phenytoin.

**Dissolution** Being specified separately.

**Assay** Weigh accurately an amount of Phenytoin Powder, equivalent to about 50 mg of phenytoin (C₁₅H₁₂N₂O₂), add 30 mL of methanol, treat with ultrasonic waves for 15 minutes with occasional shaking, shake for another 10 minutes, and add methanol to make exactly 50 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of phenytoin for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 5 mL of the
Phenytoin Tablets
Diphenylhydantoin Tablets

Phenytoin Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of phenytoin (C15H12N2O2; 252.27).

Method of preparation Prepare as directed under Tablets, with Phenytoin.

Identification Weigh a portion of powdered Phenytoin Tablets, equivalent to about 0.3 g of Phenytoin according to the labeled amount, transfer to a separator, and add 1 mL of dilute hydrochloric acid and 10 mL of water. Extract with 100 mL of diethyl ether, then with four 25-mL portions of diethyl ether. Combine the extracts, evaporate the diethyl ether on a water bath, and dry the residue at 105°C for 2 hours. Proceed with the residue as directed in the Identification under Phenytoin.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Phenytoin Tablets add 3 V/5 mL of a mixture of water and acetonitrile (1:1), treat with ultrasonic waves for 15 minutes with occasional shaking, shake for another 10 minutes, and add a mixture of water and acetonitrile (1:1) to make exactly 50 mL so that each mL contains about 1 mg of phenytoin (C15H12N2O2). Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of phenytoin (C15H12N2O2) = M5 × Q1/Q0 × V/25

M5: Amount (mg) of phenytoin for assay

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 25,000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 258 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecy(decylsilanized silica gel for liquid chromatography (5 µm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of methanol and 0.02 mol/L phosphate buffer solution, pH 3.5 (11:9).
Flow rate: Adjust the flow rate so that the retention time of phenytoin is about 5 minutes.

System suitability—
System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, phenytoin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.
System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of phenytoin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

JP XVI
Phenytoin Sodium for Injection

Diphenylhydantoin Sodium for Injection

Phenytoin Sodium for Injection occurs as white crystals or crystalline powder. It is odorless. It is soluble in water and in ethanol (95), and practically insoluble in chloroform and in diethyl ether. The pH of a solution of Phenytoin Sodium for Injection (1 in 20) is about 12. It is hygroscopic.

Phenytoin Sodium for Injection is a preparation for injection which is dissolved before use.

When dried, it contains not less than 98.5% of phenytoin sodium (C\textsubscript{15}H\textsubscript{12}N\textsubscript{2}O\textsubscript{2}), and contains not less than 92.5% and not more than 102.0% of the labeled amount of phenytoin sodium (C\textsubscript{15}H\textsubscript{11}N\textsubscript{2}NaO\textsubscript{2}).

**Method of preparation** Prepare as directed under Injection.

**Description** Phenytoin Sodium for Injection occurs as white crystals or crystalline powder. It is odorless. It is soluble in water and in ethanol (95), and practically insoluble in chloroform and in diethyl ether.

The pH of a solution of Phenytoin Sodium for Injection (1 in 20) is about 12.

It is hygroscopic.

A solution of Phenytoin Sodium for Injection absorbs carbon dioxide gradually when exposed to air, and a crystalline precipitate of phenytoin is produced.

**Identification** (1) With the residue obtained in the Assay, proceed as directed in the Identification under Phenytoin.

(2) Ignite 0.5 g of Phenytoin Sodium for Injection, cool, and dissolve the residue in 10 mL of water: the solution changes red litmus paper to blue, and responds to Qualitative Tests <1.09> (1) for sodium salt.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Phenytoin Sodium for Injection in 20 mL of freshly boiled and cooled water in a glass-stoppered test tube: the solution is clear and colorless. If any turbidity is produced, add 4.0 mL of 0.1 mol/L sodium hydroxide VS: the solution becomes clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Phenytoin Sodium for Injection according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Containers and storage** Containers—Well-closed containers.

Phenytoin Sodium for Injection for Injection which is dissolved before use.

Phenytoin Sodium for Injection occurs as white crystals or crystalline powder. It is odorless. It is soluble in water and in ethanol (95), and practically insoluble in chloroform and in diethyl ether. The pH of a solution of Phenytoin Sodium for Injection (1 in 20) is about 12. It is hygroscopic.

**Identification** (1) With the residue obtained in the Assay, proceed as directed in the Identification under Phenytoin.

(2) Ignite 0.5 g of Phenytoin Sodium for Injection, cool, and dissolve the residue in 10 mL of water: the solution changes red litmus paper to blue, and responds to Qualitative Tests <1.09> (1) for sodium salt.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Phenytoin Sodium for Injection in 20 mL of freshly boiled and cooled water in a glass-stoppered test tube: the solution is clear and colorless. If any turbidity is produced, add 4.0 mL of 0.1 mol/L sodium hydroxide VS: the solution becomes clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Phenytoin Sodium for Injection according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Containers and storage** Containers—Well-closed containers.

Phenytoin Sodium for Injection

Diphenylhydantoin Sodium for Injection

Phenytoin Sodium for Injection occurs as white crystals or crystalline powder. It is odorless. It is soluble in water and in ethanol (95), and practically insoluble in chloroform and in diethyl ether. The pH of a solution of Phenytoin Sodium for Injection (1 in 20) is about 12. It is hygroscopic.

Phenytoin Sodium for Injection is a preparation for injection which is dissolved before use.

When dried, it contains not less than 98.5% of phenytoin sodium (C\textsubscript{15}H\textsubscript{12}N\textsubscript{2}O\textsubscript{2}), and contains not less than 92.5% and not more than 102.0% of the labeled amount of phenytoin sodium (C\textsubscript{15}H\textsubscript{11}N\textsubscript{2}NaO\textsubscript{2}).

**Method of preparation** Prepare as directed under Injection.

**Description** Phenytoin Sodium for Injection occurs as white crystals or crystalline powder. It is odorless. It is soluble in water and in ethanol (95), and practically insoluble in chloroform and in diethyl ether. The pH of a solution of Phenytoin Sodium for Injection (1 in 20) is about 12. It is hygroscopic.

**Identification** (1) With the residue obtained in the Assay, proceed as directed in the Identification under Phenytoin.

(2) Ignite 0.5 g of Phenytoin Sodium for Injection, cool, and dissolve the residue in 10 mL of water: the solution changes red litmus paper to blue, and responds to Qualitative Tests <1.09> (1) for sodium salt.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Phenytoin Sodium for Injection in 20 mL of freshly boiled and cooled water in a glass-stoppered test tube: the solution is clear and colorless. If any turbidity is produced, add 4.0 mL of 0.1 mol/L sodium hydroxide VS: the solution becomes clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Phenytoin Sodium for Injection according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Containers and storage** Containers—Well-closed containers.

Phenytoin Sodium for Injection

Diphenylhydantoin Sodium for Injection

Phenytoin Sodium for Injection occurs as white crystals or crystalline powder. It is odorless. It is soluble in water and in ethanol (95), and practically insoluble in chloroform and in diethyl ether. The pH of a solution of Phenytoin Sodium for Injection (1 in 20) is about 12. It is hygroscopic.

Phenytoin Sodium for Injection is a preparation for injection which is dissolved before use.

When dried, it contains not less than 98.5% of phenytoin sodium (C\textsubscript{15}H\textsubscript{12}N\textsubscript{2}O\textsubscript{2}), and contains not less than 92.5% and not more than 102.0% of the labeled amount of phenytoin sodium (C\textsubscript{15}H\textsubscript{11}N\textsubscript{2}NaO\textsubscript{2}).

**Method of preparation** Prepare as directed under Injection.

**Description** Phenytoin Sodium for Injection occurs as white crystals or crystalline powder. It is odorless. It is soluble in water and in ethanol (95), and practically insoluble in chloroform and in diethyl ether. The pH of a solution of Phenytoin Sodium for Injection (1 in 20) is about 12. It is hygroscopic.

**Identification** (1) With the residue obtained in the Assay, proceed as directed in the Identification under Phenytoin.

(2) Ignite 0.5 g of Phenytoin Sodium for Injection, cool, and dissolve the residue in 10 mL of water: the solution changes red litmus paper to blue, and responds to Qualitative Tests <1.09> (1) for sodium salt.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Phenytoin Sodium for Injection in 20 mL of freshly boiled and cooled water in a glass-stoppered test tube: the solution is clear and colorless. If any turbidity is produced, add 4.0 mL of 0.1 mol/L sodium hydroxide VS: the solution becomes clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Phenytoin Sodium for Injection according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Containers and storage** Containers—Well-closed containers.

Phenytoin Sodium for Injection

Diphenylhydantoin Sodium for Injection

Phenytoin Sodium for Injection occurs as white crystals or crystalline powder. It is odorless. It is soluble in water and in ethanol (95), and practically insoluble in chloroform and in diethyl ether. The pH of a solution of Phenytoin Sodium for Injection (1 in 20) is about 12. It is hygroscopic.

Phenytoin Sodium for Injection is a preparation for injection which is dissolved before use.

When dried, it contains not less than 98.5% of phenytoin sodium (C\textsubscript{15}H\textsubscript{12}N\textsubscript{2}O\textsubscript{2}), and contains not less than 92.5% and not more than 102.0% of the labeled amount of phenytoin sodium (C\textsubscript{15}H\textsubscript{11}N\textsubscript{2}NaO\textsubscript{2}).

**Method of preparation** Prepare as directed under Injection.

**Description** Phenytoin Sodium for Injection occurs as white crystals or crystalline powder. It is odorless. It is soluble in water and in ethanol (95), and practically insoluble in chloroform and in diethyl ether. The pH of a solution of Phenytoin Sodium for Injection (1 in 20) is about 12. It is hygroscopic.

**Identification** (1) With the residue obtained in the Assay, proceed as directed in the Identification under Phenytoin.

(2) Ignite 0.5 g of Phenytoin Sodium for Injection, cool, and dissolve the residue in 10 mL of water: the solution changes red litmus paper to blue, and responds to Qualitative Tests <1.09> (1) for sodium salt.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Phenytoin Sodium for Injection in 20 mL of freshly boiled and cooled water in a glass-stoppered test tube: the solution is clear and colorless. If any turbidity is produced, add 4.0 mL of 0.1 mol/L sodium hydroxide VS: the solution becomes clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Phenytoin Sodium for Injection according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Containers and storage** Containers—Well-closed containers.

Phenytoin Sodium for Injection
Refractive index \( <2.45 \)  
\( n_\text{D}^2: 1.525 - 1.529 \)

**Purity (1)** Ratio of absorbances—Determine the absorbances, \( A_1 \), \( A_2 \) and \( A_3 \), of a solution of Phytonadione in isooctane (1 in 100,000) at 248.5 nm, 253.5 nm and 269.5 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry \( <2.2> \): the ratio \( A_2/A_1 \) is between 0.69 and 0.73, and the ratio \( A_2/A_3 \) is between 0.74 and 0.78. Determine the absorbances, \( A_4 \) and \( A_5 \), of a solution of Phytonadione in isooctane (1 in 10,000) at 284.5 nm and 326 nm, respectively: the ratio \( A_4/A_3 \) is between 0.28 and 0.34.

(2) Heavy metals \( <1.07> \)—Carbonize 1.0 g of Phytonadione by gentle heating. Cool, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and ignite the ethanol to burn. Cool, add 1 mL of sulfuric acid, magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and proceed according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Menadione—Dissolve 20 mg of Phytonadione in 0.5 mL of a mixture of water and ethanol (95) (1:1), add 1 drop of a solution of 3-methyl-1-phenyl-5-pyrazolone in ethanol (95) (1 in 20) and 1 drop of ammonia solution (28), and allow to stand for 2 hours: no blue-purple color develops.

**Isomer ratio** Conduct this procedure rapidly and without exposure to light. Dissolve 30 mg of Phytonadione in 50 mL of the mobile phase. To 4 mL of this solution add the mobile phase to make 25 mL. To 10 mL of this solution add the mobile phase to make 25 mL, and use this solution as the sample solution. Perform the test with 50 \( \mu \)L of the sample solution as directed under Liquid Chromatography \( <2.01> \) according to the following conditions, and determine the peak areas of \( Z \)-isomer and \( E \)-isomer, \( A_{TZ} \) and \( A_{TE} \): \( A_{TZ}/(A_{TZ} + A_{TE}) \) is between 0.05 and 0.18.

**Operating conditions**—Procedures as directed in the operating conditions in the Assay.

**System suitability**—

- System performance: When the procedure is run with 50 \( \mu \)L of the standard solution under the above operating conditions, \( Z \)-isomer and \( E \)-isomer are eluted in this order with the resolution between the peaks being not less than 1.5.
- System repeatability: When the test is repeated 6 times with 50 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the total area of the peaks of \( Z \)-isomer and \( E \)-isomer is not more than 2.0%.

**Assay** Conduct this procedure rapidly and without exposure to light. Weigh accurately about 30 mg each of Phytonadione and Phytonadione RS, and dissolve each in the mobile phase to make exactly 50 mL. Pipet 4 mL each of these solutions, and add the mobile phase to make exactly 25 mL. To exactly 10 mL each of these solutions add exactly 7 mL of the internal standard solution and the mobile phase to make 25 mL, and use these as the sample solution and the standard solution, respectively. Perform the test with 50 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.01> \) according to the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_5 \), of the total area of the peaks of \( Z \)-isomer and \( E \)-isomer to the peak area of the internal standard.

\[
\text{Amount (mg) of C}_{31}\text{H}_{46}\text{O}_2 = M_5 \times Q_1/Q_5
\]

**Internal standard solution**—A solution of cholesterol benzoate in the mobile phase (1 in 400).

**Operating conditions**—

- Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with porous silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).
- Column temperature: A constant temperature of about 30°C.
- Mobile phase: A mixture of hexane and \( n \)-amyl alcohol (4000 : 3).
- Flow rate: Adjust the flow rate so that the retention time of the peak of \( E \)-isomer of phytonadione is about 25 minutes.

**System suitability**—

- System performance: When the procedure is run with 50 \( \mu \)L of the standard solution under the above operating conditions, the internal standard, \( Z \)-isomer and \( E \)-isomer are eluted in this order with the resolution between the peaks of \( Z \)-isomer and \( E \)-isomer being not less than 1.5.
- System repeatability: When the test is repeated 6 times with 50 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the total area of the peaks of \( Z \)-isomer and \( E \)-isomer to the peak area of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.

- Storage—Light-resistant, at a cold place or in containers in which air has been displaced by Nitrogen.

### Pilocarpine Hydrochloride

ピロカルピン塩酸塩

\[
\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_2 \cdot \text{HCl} : 244.72
\]

(35,4R)-3-Ethyl-4-(1-methyl-1H-imidazol-5-ylmethyl)-4,5-dihydrofuran-2(3H)-one monohydrochloride [54-71-7]

Pilocarpine Hydrochloride, when dried, contains not less than 99.0% of \( C_{11}H_{16}N_2O_2 \cdot HCl \).

**Description** Pilocarpine Hydrochloride occurs as colorless crystals or white powder. It is odorless, and has a slightly bitter taste.

- It is very soluble in acetic acid (100), freely soluble in water, in methanol and in ethanol (95), soluble in acetic anhydride, and practically insoluble in diethyl ether.
- The \( pH \) of a solution of Pilocarpine Hydrochloride (1 in 10) is between 3.5 and 4.5.
- It is hygroscopic.
- It is affected by light.

**Identification (1)** Dissolve 0.1 g of Pilocarpine Hydrochloride in 5 mL of water, add 1 drop of dilute nitric acid, 1 mL of hydrogen peroxide TS, 1 mL of chloroform and 1 drop of a potassium dichromate solution (1 in 300), and...
shake the mixture vigorously: a violet color develops in the chloroform layer while no color or a light yellow color is produced in the aqueous layer.

(2) To 1 mL of a solution of Pilocarpine Hydrochloride (1 in 20) add 1 mL of dilute nitric acid and 2 to 3 drops of silver nitrate TS: a white precipitate or opalescence is produced.

Melting point $<2.60°$ 200 – 203°C

Purity (1) Sulfate—Dissolve 0.5 g of Pilocarpine Hydrochloride in 20 mL of water, and use this solution as the sample solution. To 5.0 mL of the sample solution add 1 mL of dilute hydrochloric acid and 0.5 mL of barium chloride TS: no turbidity is produced.

(2) Nitrate—To 2.0 mL of the sample solution obtained in (1) add 2 mL of iron (II) sulfate TS, and superimpose the mixture upon 4 mL of sulfuric acid: no dark brown color develops at the zone of contact.

(3) Related substances—Dissolve 0.3 g of Pilocarpine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.05>$. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia TS (85:14:2) to a distance of about 13 cm, and dry the plate at 105°C for 10 minutes. Cool, and spray evenly bismuth potassium iodide TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(4) Readily carbonizable substances $<1.15>$.—Take 0.25 g of Pilocarpine Hydrochloride, and perform the test: the solution has no more color than Matching Fluid B.

Loss on drying $<2.41>$ Not more than 3.0% (1 g, 105°C, 2 hours).

Residue on ignition $<2.44>$ Not more than 0.5% (0.1 g).

Assay Weigh accurately about 0.5 g of Pilocarpine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate $<2.50>$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 24.47 mg of C$_{11}$H$_{16}$N$_2$O$_2$·HCl

Containers and storage Containers—Tight containers. Storage—Light-resistant.

### Pimaricin

**Natamycin**

![Natamycin](image)

C$_{33}$H$_{47}$NO$_{13}$: 665.73

(1$\text{R}^*$,3$\text{S}^*$,5$\text{R}^*$,7$\text{R}^*$,8$\text{E}$,12$\text{R}^*$,14$\text{E}$,16$\text{E}$,18$\text{E}$,20$\text{E}$,22$\text{R}^*$,24$\text{S}^*$,25$\text{R}^*$,26$\text{S}^*$)-22-(3-Amino-3,6-dideoxy-$\beta$-d-mannopyranosyl)-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo[22.3.1.0$_{5,7}$]octacosa-8,14,16,18,20-pentaene-25-carboxylic acid [7681-93-8]

Pimaricin is a polyene macrolide substance having antifungal activity produced by the growth of Streptomyces natalensis.

It contains not less than 900 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Pimaricin is expressed as mass (potency) of pimaricin (C$_{33}$H$_{47}$NO$_{13}$).

Description Pimaricin occurs as white to yellowish white crystalline powder.

It is slightly soluble in methanol and in acetic acid (100), and practically insoluble in water and in ethanol (99.5).

Identification (1) To 3 mg of Pimaricin add 1 mL of hydrochloric acid, and mix: a blue-purple color appears.

(2) Dissolve 5 mg of Pimaricin in a solution of acetic acid (100) in methanol (1 in 100) to make 1000 mL. Determine the absorption spectrum of this solution as directed under UV-visible Spectrophotometry $<2.24>$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pimaricin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Optical rotation $<2.49>$ $[\alpha]_D^{25}$: +243 – +259° (0.1 g, acetic acid (100), 25 mL, 100 mm).

### Purity

(1) Heavy metals $<1.07>$.—Proceed with 1.0 g of Pimaricin according to Method 4, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 20 mg of Pimaricin in methanol to make 100 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography $<2.01>$. According to the following conditions, and determine the total area of the peaks other than pimaricin by the automatic integration method: not more than 4.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-
Pimozide

ピモジド

\[
C_{28}H_{29}F_{2}N_{3}O: 461.55
1-[1-[4,4-Bis(4-fluorophenyl)butyl]piperidin-4-yl]-1,3-dihydro-2H-benzoimidazol-2-one
\]

Pimozide contains not less than 98.5% and not more than 101.0% of \( C_{28}H_{29}F_{2}N_{3}O \).

**Description** Pimozide occurs as a white to pale yellowish white powder. It is freely soluble in acetic acid (100), slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

**Identification** (1) Determine the absorption spectrum of a solution of Pimozide in methanol (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pimozide as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25>\), and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** \(<2.60>\) 216 – 220°C

**Purity** (1) Heavy metals \(<1.07>\)—Proceed with 2.0 g of Pimozide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution by using 5 mL of sulfuric acid (not more than 10 ppm).

(2) Arsenic \(<1.11>\)—Prepare the test solution with 1.0 g of Pimozide according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Pimozide in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak other than the peak of pimozide from the sample solution is not larger than the peak area of pimozide from the standard solution, and the total area of the peaks other than the peak of pimozide from the sample solution is not larger than 1.5 times the peak area of pimozide from the standard solution.

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilsilicized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 2.5 g of ammonium acetate and 8.5 g of tetrabutylammonium hydrogensulfate in water to make 1000 mL.

Mobile phase B: Acetonitrile.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10</td>
<td>80 → 70</td>
<td>20 → 30</td>
</tr>
<tr>
<td>10 – 15</td>
<td>70</td>
<td>30</td>
</tr>
</tbody>
</table>

Flow rate: 2.0 mL per minute.

Time span of measurement: 1.5 times as long as the retention time of pimozoide.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 10 mL. Confirm that the peak area of pimozoide obtained from 10 μL of this solution is equivalent to 8 to 12% of that of pimozoide from 10 μL of the standard solution.

System performance: Dissolve 5 mg of Pimozoide and 2 mg of mebendazole in methanol to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, mebendazole and pimozoide are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pimozoide is not more than 2.0%.

(4) Residual solvent—Being specified separately.

Loss on drying 2.41 Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition 2.44 Not more than 0.1% (1 g).

Assay Weigh accurately about 70 mg of Pimozoide, previously dried, dissolve in 25 mL of acetic acid for nonaqueous titration, and titrate 2.50 with 0.02 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS = 9.231 mg of C14H20N2O2

Containers and storage Containers—Well-closed containers.

Pindolol

ピンドロール

C14H20N2O2: 248.32
(2RS)-1-(1H-Indol-4-yloxy)-3-(1-methylethyl)aminopropan-2-ol
[13323-86-9]

Pindolol, when dried, contains not less than 98.5% of C14H20N2O2.

Description Pindolol occurs as a white, crystalline powder. It has a slight, characteristic odor.

It is sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It dissolves in dilute sulfuric acid and in acetic acid (100).

Identification (1) To 1 mL of a solution of Pindolol in methanol (1 in 10,000) add 1 mL of a solution of 1-(4-pyridyl)-pyridinium chloride hydrochloride (1 in 1000) and 1 mL of sodium hydroxide TS, then add 1 mL of hydrochloric acid: a blue to blue-purple color, changing to red-purple, is produced.

(2) Dissolve 0.05 g of Pindolol in 1 mL of dilute sulfuric acid, and add 1 mL of Reinecke salt TS: a light red precipitate is produced.

(3) Determine the absorption spectrum of Pindolol in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Pindolol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance 2.24 E1 cm (264 nm): 333 – 350 (10 mg, methanol, 500 mL).

Melting point 2.60 169 – 173°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Pindolol in 10 mL of acetic acid (100), and observe immediately: the solution is clear, and has no more color than the following control solution.

Control solution: Measure accurately 4 mL of Matching Fluid A, add exactly 6 mL of water, and mix.

(2) Heavy metals 1.07 Proceed with 1.0 g of Pindolol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic 1.11 Prepare the test solution with 1.0 g of Pindolol according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Pindolol in 10 mL of methanol, and use this solution as the sample solu-
tion. Pipet 2 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Ultraviolet–solutions of Pioglitazone Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet–solution of Pioglitazone Hydrochloride in 0.1 mol/L hydrochloric acid TS.

**Identification**

Determine the absorption spectrum of a solution of Pioglitazone Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet–solution of Pioglitazone Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet–solution of Pioglitazone Hydrochloride in 0.1 mol/L hydrochloric acid TS. Develop the plate with a mixture of chloroform, acetone and isopropylamine (5:4:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (3 in 5) and a sodium nitrite solution (1 in 50) on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying**

Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition**

Not more than 0.1% (1 g).

**Assay**

Weigh accurately about 0.5 g of Pindolol, previously dried, dissolve in 80 mL of methanol, and titrate with 0.1 mol/L hydrochloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

\[
\text{Each mL of 0.1 mol/L hydrochloric acid VS} = 24.83 \text{ mg of C}_1\text{H}\text{H}_\text{N}_\text{O}_\text{HCl}.
\]

**Containers and storage**

Containers—Tight containers. Storage—Light-resistant.

## Pioglitazone Hydrochloride

### Description

Pioglitazone Hydrochloride occurs as white crystals or crystalline powder.

It is soluble in \(N,N\)-dimethylformamide and in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

It dissolves in 0.1 mol/L hydrochloric acid TS.

A solution of Pioglitazone Hydrochloride in \(N,N\)-dimethylformamide (1 in 20) shows no optical rotation.

### Identification

**(1)** Determine the absorption spectrum of a solution of Pioglitazone Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet–visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pioglitazone Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Pioglitazone Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Pioglitazone Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity**

*(1)* Heavy metals

Proceed with 1.0 g of Pioglitazone Hydrochloride according to Method 4, and perform the test. After incineration, use 3 mL of hydrobromic acid instead of 3 mL of hydrochloric acid. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

**(2)** Related substances

Dissolve 20 mg of Pioglitazone Hydrochloride in 20 mL of methanol, add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 40 \(\mu\)L each of the sample solution and standard solution as directed under Liquid Chromatography. Determine each peak area of both solutions by the automatic integration method: the area of the peaks, having the relative retention times of about 0.7, about 1.4 and about 3.0 with respect to pioglitazone from the sample solution, is not larger than 2/5 times the peak area of pioglitazone from the standard solution, and the area of each peak other than the peak of pioglitazone and other than those mentioned above is smaller than 1/5 times the peak area of pioglitazone from the standard solution. Furthermore, the total area of the peaks other than the peak of pioglitazone is not larger than the peak area of pioglitazone from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of pioglitazone, beginning after the solvent peak.

**System suitability**

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of pioglitazone obtained from 40 \(\mu\)L of this solution is equivalent to 7 to 13\% of that of pioglitazone from 40 \(\mu\)L of the standard solution.

System performance: Dissolve 50 mg of Pioglitazone Hydrochloride in 10 mL of a solution of benzophenone in methanol (1 in 750), and add methanol to make 100 mL. To 1 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 40 \(\mu\)L of this solution under the above operating conditions, pioglitazone and benzophenone are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 40 \(\mu\)L of the standard solution under the above operat-
Weigh accurately about 50 mg each of Pioglitazone Hydrochloride and Pioglitazone Hydrochloride RS separately, determine the water in the same manner as Pioglitazone Hydrochloride, add exactly 10 mL of the internal standard solution and methanol to make 100 mL. Pipet 2 mL each of these solutions, add the mobile phase to make 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.0 according to the following conditions, and calculate the ratios, Q1 and Q2, of the peak area of pioglitazone to that of the internal standard.

\[
M_S = M_S \times \frac{Q_1}{Q_3}
\]

**Internal standard solution**—A solution of benzophenone in methanol (1 in 750).

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 269 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of ammonium acetate solution (77 in 10,000), acetonitrile and acetic acid (100) (25:25:1).
Flow rate: Adjust the flow rate so that the retention time of pioglitazone is about 7 minutes.

**System suitability**—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, pioglitazone and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pioglitazone is not more than 1.0%.

**Containers and storage**—Containers—Well-closed containers.
first 5 mL of the filtrate, pipet $V$ mL of the subsequent filtrate, add the dissolution medium to make exactly $V'$ mL so that each mL contains about 18 μg of pioglitazone hydrochloride (C19H20N2O3S·HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 23 mg of Pioglitazone Hydrochloride RS (separately determine the water <2.49> in the same manner as Pioglitazone Hydrochloride), dissolve in 10 mL of methanol, and add the dissolution medium to make exactly 50 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, $A_1$ and $A_3$, of the sample solution and standard solution at 269 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of pioglitazone hydrochloride (C19H20N2O3S·HCl)

\[
M_S = \frac{A_T - A_3}{V/V' \times 1/C \times 72}
\]

$M_S$: Amount (mg) of Pioglitazone Hydrochloride RS, calculated on the anhydrous basis

$C$: Labeled amount (mg) of pioglitazone hydrochloride (C19H20N2O3S·HCl) in 1 tablet

Assay Accurately weigh the mass of not less than 20 Pioglitazone Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg of pioglitazone hydrochloride (C19H20N2O3S·HCl), add 45 mL of methanol and exactly 5 mL of the internal standard solution, agitate with the aid of ultrasonic waves, and centrifuge. To 2 mL of the supernatant liquid add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Pioglitazone Hydrochloride RS (separately, determine the water <2.49> in the same manner as Pioglitazone Hydrochloride), dissolve in 45 mL of methanol, and add exactly 5 mL of the internal standard solution. Pipet 2 mL of this solution, add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.20> according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of pioglitazone to that of the internal standard.

\[
\text{Amount (mg) of pioglitazone hydrochloride} = M_S \times \frac{Q_T}{Q_S}
\]

$M_S$: Amount (mg) of Pioglitazone Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of benzophenone in methanol (1 in 750).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 269 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of ammonium acetate solution (77 in 10,000), acetonitrile and acetic acid (100) (25:25:1).

Flow rate: Adjust the flow rate so that the retention time of pioglitazone is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, pioglitazone and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pioglitazone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

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**Pipemidic Acid Hydrate**

ピペミド酸水和物

\[
\text{C}_{14}\text{H}_{17}\text{N}_5\text{O}_3\cdot3\text{H}_2\text{O}: 357.36
\]

8-Ethyl-5-oxo-2-(piperazin-1-yl)-5,8-dihydropyrido[2,3-d]pyrimidine-6-carboxylic acid trihydrate [51940-44-4, anhydride]

Pipemidic Acid Hydrate contains not less than 98.5% and not more than 101.0% of pipemidic acid (C14H17N5O3·3H2O: 303.32), calculated on the anhydrous basis.

**Description** Pipemidic Acid Hydrate occurs as a pale yellow, crystalline powder.

It is freely soluble in acetic acid (100), very slightly soluble in water and in ethanol (99.5), and practically insoluble in methanol.

It dissolves in sodium hydroxide TS.

It is gradually colored on exposure to light.

Melting point: about 250°C (with decomposition).

**Identification** (1) Dissolve 0.1 g of Pipemidic Acid Hydrate in 20 mL of sodium hydroxide TS, and dilute with water to make 200 mL. To 1 mL of the solution add water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pipemidic Acid Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** (1) Chloride <1.03>—Dissolve 1.0 g of Pipemidic Acid Hydrate in 35 mL of water and 10 mL of sodium hydroxide TS, then add 15 mL of dilute nitric acid, shake well, and filter through a glass filter (G3). To 30 mL of the filtrate...
add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS add 5 mL of sodium hydroxide TS, 13.5 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(2) Sulfate—Dissolve 1.0 g of Pipemidic Acid Hydrate in 35 mL of water and 10 mL of sodium hydroxide TS, then add 15 mL of dilute hydrochloric acid, shake well, and filter through a glass filter (G3). To 30 mL of the filtrate add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS add 5 mL of sodium hydroxide TS, 7.5 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals—Proceed with 2.0 g of Pipemidic Acid Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic—Proceed with the 1.0 g of Pipemidic Acid Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Pipemidic Acid Hydrate in 10 mL of dilute acetic acid (100) (1 in 20), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dilute acetic acid (100) (1 in 20) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, formic acid and triethylamine (25:15:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water—14.5 – 16.0% (20 mg, coulometric titration).

Residue on ignition—Not more than 0.1% (1 g).

Assay—Weigh accurately about 0.35 g of Pipemidic Acid Hydrate, dissolve in 40 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 30.33 mg of C8H8N2O3

Containers and storage—Containers—Well-closed containers.

Storage—Light-resistant.

### Pipacillin Hydrate

Pipacillin Hydrate contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Pipacillin Hydrate is expressed as mass (potency) of pipacillin (C_{32}H_{37}N_{2}O_{5}S: 517.55).

**Description**—Pipacillin Hydrate occurs as a white crystalline powder.

It is freely soluble in methanol, soluble in ethanol (99.5) and in dimethylsulfoxide, and very slightly soluble in water.

**Identification**

(1) Determine the infrared absorption spectrum of Pipacillin Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Pipacillin: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the 1H spectrum of a solution of Pipacillin Hydrate in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 3) as directed under Nuclear Magnetic Resonance Spectroscopy, using tetramethylsilane as an internal reference compound: it exhibits a triple signal A at about δ 1.1 ppm, a single signal B at about δ 4.2 ppm, and a multiple signal C at about δ 7.4 ppm, and the ratio of the integrated intensity of each signal, A:B:C, is about 3:1:5.

**Optical rotation**—[α]_D^20 = +162 – +172° (0.2 g, methanol, 20 mL, 100 mm).

**Purity**

(1) Heavy metal—Proceed with 2.0 g of Pipacillin Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure rapidly after the preparation of the sample solution and standard solution. Dissolve 20 mg of Pipacillin Hydrate in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solutions (1) and (2) as directed under Liquid Chromatography according to the following conditions, and...
determine each peak area by the automatic integration method: the total area of the peaks, having the relative retention time of about 0.38 and about 0.50 with respect to piperacillin, obtained from the sample solution is not larger than 2 times the peak area of piperacillin from the standard solution (2), the total area of the peaks, having the relative retention time of about 0.82 and about 0.86 with respect to piperacillin, obtained from the sample solution is not larger than the peak area of piperacillin from the standard solution (2), and the area of the peak other than piperacillin and other than the peaks having the relative retention time of about 0.38, about 0.50, about 0.82 and about 0.86 with respect to piperacillin, obtained from the sample solution, is not larger than the peak area of piperacillin from the standard solution (2). Furthermore, the total area of the peaks other than piperacillin obtained from the sample solution is not larger than the peak area of piperacillin from the standard solution (1).

Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of piperacillin, beginning after the solvent peak.

System suitability—
Test for required detectability: Confirm that the peak area of piperacillin obtained from 20 μL of the standard solution (2) is equivalent to 15 to 25% of that from 20 μL of the standard solution (1).

System performance: When the procedure is run with 20 μL of the standard solution (1) under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of piperacillin are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the peak area of piperacillin is not more than 3.0%.

(3) Related substances 2—Dissolve 20 mg of Piperacillin Hydrate in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution (1). Pipet 2 mL of the standard solution (1), add the mobile phase to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 20 μL each of the sample solution, and the standard solutions (1) and (2) as directed under Liquid Chromatography 2.01) according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 6.6 with respect to piperacillin, obtained from the sample solution is not larger than 3 times the peak area of piperacillin from the standard solution (2), and the area of the peaks other than the peak of piperacillin and the peak having the relative retention time of about 6.6 with respect to piperacillin from the sample solution are not larger than 1.4 times the peak area of piperacillin from the standard solution (2). Furthermore, the total area of the peaks other than the peak of piperacillin from the sample solution is not larger than the area of the peak of piperacillin from the standard solution (1). For these calculations, use the area of the peak, having the relative retention time of about 6.6 with respect to piperacillin, after multiplying by the relative response factor, 2.0.

Operating conditions—
Detector, column and column temperature: Proceed as directed in the operating conditions in the Assay.
Mobile phase: Take 60.1 g of acetic acid (100) and 101.0 g of triethylamine, add water to make 1000 mL. To 25 mL of this solution add 300 mL of acetonitrile and 25 mL of dilute acetic acid, and add water to make 1000 mL.
Flow rate: Adjust the flow rate so that the retention time of piperacillin is about 1.2 minutes.
Time span of measurement: About 8 times as long as the retention time of piperacillin, beginning after the piperacillin peak.

System suitability—
Test for required detectability: Confirm that the peak area of piperacillin obtained from 20 μL of the standard solution (2) is equivalent to 15 to 25% of that from 20 μL of the standard solution (1).
System performance: When the procedure is run with 20 μL of the standard solution (1) under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of piperacillin are not less than 1500 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the peak area of piperacillin is not more than 4.0%.

(4) Residual solvents 2.46—Transfer exactly 10 mg of Piperacillin Hydrate to an about 3 mL-vial, add exactly 1 mL of saturated sodium hydrogen carbonate solution to dissolve and stop the vial tightly. After heating this at 90°C for 10 minutes, use the gas inside the container as the sample gas. Separately, measure exactly 1 mL of ethyl acetate, dissolve in water to make exactly 200 mL. Pipet 10 mL of this solution, add water to make exactly 20 mL. Pipet 2 μL of this solution in an about 3 mL-vial containing exactly 1 mL of saturated sodium hydrogen carbonate solution, and stop the vial tightly. Run the procedure similarly to the sample, and use the gas as the standard gas. Perform the test with exactly 0.5 mL each of the sample gas and standard gas as directed under Gas Chromatography 2.02) according to the following conditions, and determine the peak area of ethyl acetate by the automatic integration method: the peak area of ethyl acetate obtained from the sample gas is not larger than that from the standard gas.

Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A glass column 3 mm in inside diameter and 1 m in length, packed with porous stylene-divinyl benzene copolymer for gas chromatography (average pore diameter of 0.0085 μm, 300 – 400 m2/g) with the particle size of 125 to 150 μm.
Column temperature: A constant temperature of about 145°C.
Carrier gas: Nitrogen.
Flow rate: Adjust the flow rate so that the retention time of ethyl acetate is about 4 minutes.

System suitability—
System performance: Take 1 mL of saturated sodium hydrogen carbonate solution in an about 3 mL-vial, add 2 μL each of ethyl acetate solution (1 in 400) and acetone solution (1 in 400), and stop the vial tightly. When the procedure
is run under the above operating conditions, acetone and ethyl acetate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: Take 1 mL of saturated sodium hydrogen carbonate solution in about 3 mL-vial, add 2 μL of ethyl acetate solution (1 in 400), stop the vial tightly, and perform the test under the above operating conditions. When the procedure is repeated 6 times, the relative standard deviation of the peak area of ethyl acetate is not more than 10%.

**Water** $< 2.48$ Not less than 3.2% and not more than 3.8% (0.5 g, volumetric titration, direct titration).

**Residue on ignition** $< 2.48$ Not more than 0.1% (1 g).

**Bacterial endotoxins** $< 4.01$ Less than 0.07 EU/mg (potency).

**Assay** Weigh accurately an amount of Piperacillin Hydrate and Piperacillin RS, equivalent to about 50 mg (potency), dissolve each in the mobile phase to make exactly 50 mL.

Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography $< 2.01$ according to the following conditions, and calculate the ratios, $H_1$ and $H_4$, of the peak height of piperacillin to that of the internal standard.

Amount [μg (potency)] of piperacillin ($C_{23}H_{27}N_5O_7S$) $= M_s \times H_4 / H_3 \times 1000$

$M_s$: Amount [mg (potency)] of Piperacillin RS

**Internal standard solution**—A solution of acetanilide in the mobile phase (1 in 5000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Take 60.1 g of acetic acid (100) and 101.0 g of triethylamine, add water to make 1000 mL. To 25 mL of this solution add 210 mL of acetonitrile and 25 mL of dilute acetic acid, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of piperacillin is about 5 minutes.

**System suitability**—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and piperacillin are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak height of piperacillin to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.
tion, the total area of related compounds 1 appeared at the retention time of about 17 minutes and about 21 minutes is not larger than 2 times of the peak area of piperacillin from the standard solution, the peak area of related compound 2 appeared at the retention time of about 56 minutes is not larger than that of piperacillin from the standard solution, and the total area of the peaks other than piperacillin is not larger than 5 times of the peak area of piperacillin from the standard solution. The peak areas of ampicillin, related compounds 1 and related compound 2 are used after multiplying by their relative response factors, 1.39, 1.32 and 1.11, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of water, acetonitrile and 0.2 mol/L potassium dihydrogenphosphate (45:4:1).

Mobile phase B: A mixture of acetonitrile, water and 0.2 mol/L potassium dihydrogenphosphate (25:24:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 7</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>7 – 13</td>
<td>100 → 83</td>
<td>0 → 17</td>
</tr>
<tr>
<td>13 – 41</td>
<td>83</td>
<td>17</td>
</tr>
<tr>
<td>41 – 56</td>
<td>83 → 20</td>
<td>17 → 80</td>
</tr>
<tr>
<td>56 – 60</td>
<td>20</td>
<td>80</td>
</tr>
</tbody>
</table>

Flow rate: 1.0 mL per minute. The retention time of piperacillin is about 33 minutes.

Time span of measurement: About 1.8 times as long as the retention time of piperacillin beginning after the solvent peak.

System suitability—

System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of piperacillin are not less than 15,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 3 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of piperacillin is not more than 2.0%.

Water <2.48> Not more than 1.0% (3 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Piperacillin Sodium, equivalent to about 0.1 g (potency), and dissolve in water to make exactly 100 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately an amount of Piperacillin RS, equivalent to about 0.1 g (potency), and dissolve in the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 5 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q1 and Q2, of the peak height of piperacillin to that of the internal standard.

Amount [µg (potency)] of piperacillin (C23H27N5O7S) = M5 × Q1/Q2 × 1000

M5: Amount [mg (potency)] of Piperacillin RS

Internal standard solution—A solution of acetanilide RS in the mobile phase (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 60.1 g of acetic acid (100) and 101.0 g of triethylamine add water to make exactly 1000 mL. To 25 mL of this solution add 25 mL of dilute acetic acid and 210 mL of acetonitrile, and add water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of piperacillin is about 5 minutes.

System suitability—

System performance: When the procedure is run with 5 µL of the standard solution under the above operating conditions, the internal standard and piperacillin are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of piperacillin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Piperacillin Sodium for Injection

注射用ピペラシリンナトリウム

Piperacillin Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of piperacillin (C23H27N5O7S: 517.55).

Method of preparation Prepare as directed under Injections, with Piperacillin Sodium.

Description Piperacillin Sodium for Injection is a white powder or masses.

Identification Piperacillin Sodium for Injection is a white powder or masses.

pH <2.54> The pH of a solution prepared by dissolving an amount of Piperacillin Sodium for Injection, equivalent to 1.0 g (potency) of Piperacillin Sodium according to the la-
babeled amount, in 4 mL of water is 5.0 - 7.0.

**Purity** (1) Clarity and color of solution—Dissolve an amount of Piperacillin Sodium for Injection, equivalent to 4.0 g (potency) of Piperacillin Sodium according to the labeled amount, in 17 mL of water: the solution is clear and colorless.

(2) Related substances—Proceed as directed in the Purity (4) under Piperacillin Sodium.

**Water** <2.48> Not more than 1.0% (3 g, volumetric titration, direct titration).

**Bacterial endotoxins** <4.01> Less than 0.04 EU/mg (potency).

**Uniformity of dosage units** <6.02> It meets the requirement of the Mass variation test.

**Foreign insoluble matter** <6.06> Perform the test according to Method 2: it meets the requirement.

**Insoluble particulate matter** <6.07> It meets the requirement.

**Sterility** <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Weigh accurately the mass of the contents of not less than 10 Piperacillin Sodium for Injection. Weigh accurately an amount of the contents, equivalent to about 20 mg (potency) of Piperacillin Sodium, dissolve in water to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 20 mg (potency) of Piperacillin RS, and dissolve in the mobile phase to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Proceed as directed in the Assay under Piperacillin Sodium.

\[
\text{Amount [mg (potency)] of piperacillin (C}_{23}\text{H}_{27}\text{N}_{5}\text{O}_{7}\text{S}) = M_z \times Q_Q / Q_S
\]

\[
M_z: \text{Amount [mg (potency)] of Piperacillin RS}
\]

**Internal standard solution**—A solution of acetanilide in the mobile phase (1 in 5000).

**Containers and storage** Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

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**Piperazine Adipate**

ピペラジンアジピン酸塩

\[
\text{C}_{6}\text{H}_{10}\text{N}_{2}\cdot \text{C}_{6}\text{H}_{10}\text{O}_{4}: 232.28
\]

Piperazine hexanedioate

[142-88-1]

Piperazine Adipate, when dried, contains not less than 98.5% of C_{6}H_{10}N_{2}·C_{6}H_{10}O_{4}.

**Description** Piperazine Adipate occurs as a white, crystal-line powder. It is odorless, and has a slightly acid taste.

It is soluble in water and in acetic acid (100), and practically insoluble in ethanol (95), in acetone and in diethyl ether.

Melting point: about 250°C (with decomposition).

**Identification** (1) Dissolve 0.5 g of Piperazine Adipate in 10 mL of water, add 1 mL of hydrochloric acid, and extract with two 20-mL portions of diethyl ether. Combine the diethyl ether extracts, evaporate to dryness on a water bath, and dry the residue at 105°C for 1 hour: the melting point <2.60> is between 152°C and 155°C.

(2) To 3 mL of a solution of Piperazine Adipate (1 in 100) add 3 drops of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the infrared absorption spectrum of Piperazine Adipate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**pH** <2.54> The pH of a solution of Piperazine Adipate (1 in 20) is between 5.0 and 6.0.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Piperazine Adipate in 30 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Piperazine Adipate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.2 g of Piperazine Adipate, previously dried, dissolve in a mixture of 20 mL of acetic acid for nonaqueous titration and 40 mL of acetone for nonaqueous titration, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the red-purple color of the solution changes to blue-purple (indicator: 6 drops of bromocresol green-methylrosaniline chloride TS). Perform a blank determination, and make any necessary correction.

\[
\text{Each mL of 0.1 mol/L perchloric acid VS} = 11.61 \text{ mg of C}_{6}\text{H}_{10}\text{N}_{2}\cdot \text{C}_{6}\text{H}_{10}\text{O}_{4}
\]

**Containers and storage** Containers—Well-closed containers.

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**Piperazine Phosphate Hydrate**

ピペラジンリン酸塩水和物

\[
\text{C}_{6}\text{H}_{10}\text{N}_{2}\cdot \text{H}_{3}\text{PO}_{4} \cdot \text{H}_{2}\text{O}: 202.15
\]

Piperazine monophosphate monohydrate

[18534-18-4]

Piperazine Phosphate Hydrate contains not less
than 98.5% of piperazine phosphate (C₄H₁₀N₂.H₂PO₄: 184.13), calculated on the anhydrous basis.

**Description**  Piperazine Phosphate Hydrate occurs as white crystals or crystalline powder. It is odorless, and has a slightly acid taste.

- It is soluble in formic acid, sparingly soluble in water, very slightly soluble in acetic acid (100), and practically insoluble in methanol, in ethanol (95) and in diethyl ether.
- It dissolves in dilute hydrochloric acid.
- Melting point: about 222°C (with decomposition).

**Identification**  (1) To 3 mL of a solution of Piperazine Phosphate Hydrate (1 in 100) add 3 drops of Reinecke salt TS: a light red precipitate is formed.

(2) Determine the infrared absorption spectrum of Piperazine Phosphate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Piperazine Phosphate Hydrate (1 in 100) responds to Qualitative Tests <1.09> (1) and (3) for phosphate.

**pH** <2.54> Dissolve 1.0 g of Piperazine Phosphate Hydrate in 100 mL of water: the pH of the solution is between 6.0 and 6.5.

**Purity**  (1) Chloride <1.07>—To 0.5 g of Piperazine Phosphate Hydrate add 5 mL of dilute nitric acid and water to make 50 mL. Use this solution as the test solution, and perform the test. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(2) Heavy metals <1.07>—To 2.0 g of Piperazine Phosphate Hydrate add 5 mL of dilute hydrochloric acid, 30 mL of water and 2 mL of dilute acetic acid, and dissolve. Add sodium hydroxide TS, adjust the pH of the solution to 3.3, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.17>—Dissolve 2.0 g of Piperazine Phosphate Hydrate in 5 mL of dilute hydrochloric acid, and use this solution as the test solution. Perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 50 mg of Piperazine Phosphate Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.08>. Spot 5 μL each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ammonia solution (28), aceton and ethanol (99.5) (8:3:3:2) to a distance of about 13 cm, and air-dry the plate. Spray evenly 4-dimethylaminoazobenzaldehyde TS, and allow to stand for 15 minutes: the spots other than the principal spot and the spot on the starting line from the sample solution are not more intense than the spot from the standard solution.

**Water** <2.48> 8.0 – 9.5% (0.3 g, volumetric titration, direct titration).

**Assay** Weigh accurately about 0.15 g of Piperazine Phosphate Hydrate, dissolve in 10 mL of formic acid, add 60 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 9.207 mg of C₄H₁₀N₂.H₂PO₄

**Containers and storage**  Containers—Well-closed containers.

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**Piperazine Phosphate Tablets**

**ピペラジンリン酸塩錠**

Piperazine Phosphate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of piperazine phosphate hydrate (C₄H₁₀N₂.H₃PO₄.H₂O: 202.15).

**Method of preparation**  Prepare as directed under Tablets, with Piperazine Phosphate Hydrate.

**Identification**  Take a quantity of Piperazine Phosphate Tablets equivalent to 0.1 g of Piperazine Phosphate Hydrate according to the labeled amount, previously powdered, add 10 mL of water, shake while warming for 10 minutes, allow to cool, and filter. To 3 mL of the filtrate add 3 drops of Reinecke salt TS: a light red precipitate is formed.

**Disintegration** <6.09>  It meets the requirement. The time limit of the test is 10 minutes.

**Assay** Weigh accurately not less than 20 Piperazine Phosphate Tablets, and powder. Weigh accurately a quantity of the powder, equivalent to about 0.15 g of piperazine phosphate hydrate (C₄H₁₀N₂.H₃PO₄.H₂O). Add 5 mL of formic acid, shake for 5 minutes, centrifuge, and collect the supernatant liquid. To the residue add 5 mL of formic acid, shake for 5 minutes, centrifuge, and collect the supernatant liquid. Repeat twice the same procedure with 5 mL each of acetic acid (100), combine all the supernatant liquids, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 10.11 mg of C₄H₁₀N₂.H₃PO₄.H₂O

**Containers and storage**  Containers—Tight containers.
Pirarubicin

ピラルビシン

\[
\text{C}_{32}\text{H}_{37}\text{NO}_{12}: \ 627.64 \\
(25,45)-4-[3\text{-Amino-2,3,6-trideoxy-4-O-[(2R)-3,4,5,6-tetrahydro-2H-pyran-2-yl]-L-lyxo-hexopyranosyloxy}]2,5,12\text{-trihydroxy-2-hydroxyacetyl}-1,2,3,4-tetrahydrotetracene-6,11-dione
\]

Pirarubicin is a derivative of daunorubicin. It contains not less than 950 μg (potency) per mg, calculated on the anhydrous basis. The potency of Pirarubicin is expressed as mass (potency) of pirarubicin (C_{32}H_{37}NO_{12}).

**Description** Pirarubicin occurs as a red-orange crystalline powder.

It is soluble in chloroform, very slightly soluble in acetonitrile, in methanol and in ethanol (99.5), and practically insoluble in water.

**Identification** (1) Dissolve 10 mg of Pirarubicin in 80 mL of methanol and 6 mL of diluted hydrochloric acid (1 in 5000), and add water to make 100 mL. To 10 mL of this solution add diluted methanol (4 in 5) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \(<2.49\) and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pirarubicin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 5 mg each of Pirarubicin and Pirarubicin RS in 5 mL of chloroform, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \(<2.01\). Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (5:1) to a distance of about 10 cm, and air-dry the plate. Examine the spots with the necked eye: the principal spot obtained from the sample solution and the spot from the standard solution show a red-orange color and the same Rf value.

**Optical rotation** \(<2.49> \ [\alpha]_{D}^{25}: +195 \sim +215^\circ \) (10 mg, chloroform, 10 mL, 100 mm).

**Purity** (1) Clarity and color of solution—Dissolve 10 mg of Pirarubicin in 10 mL of 0.01 mol/L hydrochloric acid TS: the solution is clear and red.

(2) Heavy metals \(<1.07\)—Proceed with 1.0 g of Pirarubicin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 10 mg of Pirarubicin in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\) according to the following conditions, and determine each peak area by the automatic integration method: the peak area of doxorubicin, having the relative retention time of about 0.45 with respect to pirarubicin, and the area of the peak, having the relative retention time of about 1.2 with respect to pirarubicin, obtained from the sample solution are not larger than the peak area of pirarubicin from the standard solution, respectively, and the sum of the areas of the peaks, having the relative retention times of about 1.9 and about 2.0 with respect to pirarubicin, from the sample solution is not larger than 5 times the peak area of pirarubicin from the standard solution. For these calculations, use the peak area for doxorubicin after multiplying by the relative response factor 0.94 and the area for the two peaks, having the relative retention times of about 1.9 and about 2.0, after multiplying by their relative response factors, 1.09, respectively.

**Operating conditions**—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of pirarubicin.

**System suitability**—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of pirarubicin obtained from 20 μL of this solution is equivalent to 14 to 26% of that from 20 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

**Water** \(<2.48>\) Not more than 2.0% (0.1 g, volumetric titration, direct titration).

**Assay** Weigh accurately an amount of Pirarubicin and Pirarubicin RS, equivalent to about 10 mg (potency), and dissolve in the mobile phase to make exactly 10 mL. Pipet 5 mL of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01\) according to the following conditions, and calculate the ratios, Q_s and Q_{Q_s}, of the peak area of pirarubicin to that of the internal standard.

\[
\text{Amount} \ [\mu g \ (\text{potency})] \ of \ C_{32}\text{H}_{37}\text{NO}_{12} = M_s \times Q_s/\bar{Q_s} \times 1000 \\
M_s: \ \text{Amount} \ [\mu g \ (\text{potency})] \ of \ Pirarubicin \ RS
\]

**Internal standard solution**—A solution of 2-naphthol in the mobile phase (1 in 1000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L ammonium formate buffer solution, pH 4.0 and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of pirarubicin is about 7 minutes.

**System suitability**

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, pirarubicin and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pirarubicin to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Hermetic containers.

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**Pirenoxine**

ピレノキシン

\[
\text{C}_{16}\text{H}_{8}\text{N}_{2}\text{O}_{5} \cdot 308.25
\]

1-Hydroxy-5-oxo-5H-pyrido[3,2-α]phenoxazine-3-carboxylic acid

[1043-21-6]

Pirenoxine, when dried, contains not less than 98.0% of \(\text{C}_{16}\text{H}_{8}\text{N}_{2}\text{O}_{5}\).

**Description** Pirenoxine occurs as a yellow-brown powder. It is odorless, and has a slightly bitter taste.

It is very slightly soluble in dimethylsulfoxide, and practically insoluble in water, in acetonitrile, in ethanol (95%), in tetrahydrofuran and in diethyl ether.

Melting point: about 250°C (with decomposition).

**Identification**

1. **Dissolve** 2 mg of Pirenoxine in 10 mL of phosphate buffer solution, pH 6.5, add 5 mL of a solution of L-ascorbic acid (1 in 50), and shake vigorously: a dark purple precipitate is formed.

2. **Determine** the absorption spectrum of a solution of Pirenoxine in phosphate buffer solution, pH 6.5 (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.2>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

3. **Determine** the infrared absorption spectrum of Pirenoxine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity**

1. **Heavy metals** — Proceed with 1.0 g of Pirenoxine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

2. **Related substances** — Dissolve 10 mg of Pirenoxine in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than pirenoxine is not larger than the peak area of pirenoxine from the standard solution.

**Operating conditions**

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 1.39 g of tetra n-butylammonium chloride and 4.5 g of disodium hydrogen phosphate dodecahydrate in 1000 mL of water, and adjust the pH to 6.5 with phosphoric acid. To 700 mL of this solution add 200 mL of acetonitrile and 30 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of pirenoxine is about 10 minutes.

Time span of measurement: About 3 times as long as the retention time of pirenoxine.

**System suitability**

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 30 mL. Confirm that the peak area of pirenoxine obtained from 5 μL of this solution is equivalent to 5 to 8 μL of that of pirenoxine obtained from 5 μL of the standard solution.

System performance: Dissolve 3 mg of Pirenoxine and 16 mg of methyl parahydroxybenzoate in 100 mL of the mobile phase. When the procedure is run with 5 μL of this solution under the above operating conditions, pirenoxine and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pirenoxine is not more than 1.0%.

**Loss on drying** — Not more than 1.5% (0.5 g, in vacuum, 80°C, 3 hours).

**Residue on ignition** — Not more than 0.1% (1 g).

**Assay**

Weigh accurately about 0.1 g of Pirenoxine, previously dried, dissolve in 140 mL of dimethylsulfoxide by heating on a water bath. After cooling, add 30 mL of water, and titrate <2.50> immediately with 0.02 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L sodium hydroxide VS = 6.165 mg of \(\text{C}_{16}\text{H}_{8}\text{N}_{2}\text{O}_{5}\).

**Containers and storage** Containers—Tight containers.
Pirenzepine Hydrochloride Hydrate

ピレンゼピン塩酸塩水和物

C₁₉H₂₁N₅O₂·2HCl·H₂O: 442.34
11-[(4-Methylpiperazin-1-yl)acetetyl]-5,11-dihydro-6H-
pyrido[2,3-b][1,4]benzodiazepin-6-one dihydrochloride
monohydrate
[29868-97-1, anhydride]

Pirenzepine Hydrochloride Hydrate contains not
less than 98.5% and not more than 101.0% of pirenze-
pine hydrochloride (C₁₉H₂₁N₅O₂·2HCl: 424.32), calcu-
lated on the anhydrous basis.

Description Pirenzepine Hydrochloride Hydrate occurs as
a white to pale yellow crystalline powder.

It is freely soluble in water and in formic acid, slightly
soluble in methanol, and very slightly soluble in ethanol
(99.5).

The pH of a solution by dissolving 1 g of Pirenzepine Hy-
drochloride Hydrate in 10 mL of water is between 1.0 and
2.0.

Melting point: about 245°C (with decomposition).

It is gradually colored by light.

Identification (1) Determine the absorption spectrum of a
solution of Pirenzepine Hydrochloride Hydrate (1 in 40,000)
as directed under Ultraviolet-visible Spectrophotometry
<2.2>, and compare the spectrum with the Reference Spec-
trum: both spectra exhibit similar intensities of absorption at
the same wavelengths.

(2) Determine the infrared absorption spectrum of Piren-
zepine Hydrochloride Hydrate as directed in the potassium
chloride disk method under Infrared Spectrophotometry
<2.25>, and compare the spectrum with the Reference Spec-
trum: both spectra exhibit similar intensities of absorption at
the same wave numbers.

(3) A solution of Pirenzepine Hydrochloride Hydrate
(1 in 50) responds to Qualitative Tests <1.09> for chloride.

Purity (1) Clarity and color of solution—A solution ob-
tained by dissolving 1.0 g of Pirenzepine Hydrochloride
Hydrate in 10 mL of water is clear and not more color than that
of the following control solution.

Control solution: To 1.2 mL of Matching fluid for color F
add 8.8 mL of diluted hydrochloric acid (1 in 40).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Pirenze-
pine Hydrochloride Hydrate according to Method 2, and
perform the test. Prepare the control solution with 2.0 mL of
Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.3 g of Pirenzepine
Hydrochloride Hydrate in 10 mL of water. To 1 mL of this
solution add 5 mL of methanol and the mobile phase A to
make 10 mL, and use this solution as the sample solution.
Pipet 1 mL of the sample solution, and add 5 mL of metha-
olin and the mobile phase A to make exactly 10 mL. Pipet 1
mL of this solution, add 5 mL of methanol and the mobile
phase A to make exactly 10 mL, and use this solution as the
standard solution. Perform the test with exactly 10 μL each
of the sample solution and standard solution as directed
under Liquid Chromatography <2.01> according to the fol-
lowing conditions, and determine each peak area by the au-
tomatic integration method: the area of the peak other than
pirenzepine is not larger than 3/5 times the peak area of
pirenzepine from the standard solution, and the total area of
the peaks other than pirenzepine is not larger than 3/5 times
the peak area of pirenzepine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-
length: 283 nm).

Column: A stainless steel column 4.6 mm in inside diame-
ter and 15 cm in length, packed with octadecylsilanized silica
gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about
40°C.

Mobile phase A: Dissolve 2 g of sodium lauryl sulfate in
900 mL of water, adjust the pH to 3.2 with acetic acid (100),
and add water to make 1000 mL.

Mobile phase B: Methanol.

Mobile phase C: Acetonitrile.

Flowing of the mobile phase: Control the gradient by mix-
ing the mobile phases A, B and C as directed in the follow-
ing table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
<th>Mobile phase C (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 15</td>
<td>55 → 25</td>
<td>30</td>
<td>15 → 45</td>
</tr>
<tr>
<td>15 -</td>
<td>25</td>
<td>30</td>
<td>45</td>
</tr>
</tbody>
</table>

Flow rate: Adjust the flow rate so that the retention time
of pirenzepine is about 8 minutes.

Time span of measurement: About 2 times as long as the
retention time of pirenzepine beginning after the solvent
peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard
solution, and add 5 mL of methanol and the mobile phase A
to make exactly 10 mL. Confirm that the peak area of piren-
zepine obtained from 10 μL of this solution is equivalent to
7 to 13% of that from 10 μL of the standard solution.

System performance: Dissolve 0.1 g of phenylpiperazine
hydrochloride in 10 mL of methanol. Mix 1 mL of this solu-
tion and 1 mL of the sample solution, and add 5 mL of
methanol and the mobile phase A to make 10 mL. When the
procedure is run with 10 μL of this solution under the above
operating conditions, pirenzepine and phenylpiperazine are
eluted in this order with the resolution between these peaks
being not less than 5.

System repeatability: When the test is repeated 6 times
with 10 μL of the standard solution under the above operat-
ing conditions, the relative standard deviation of the peak
area of pirenzepine is not more than 2.0%.

Water <2.48> Not less than 3.5% and not more than 5.0%
(0.3 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Pirenzepine Hydrochloride Hydrate, dissolve in 2 mL of formic acid, add 60 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 14.14 mg of C₁₂H₁₂N₄O₂·2HCl

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Piroxicam

ピロキシカム

C₁₅H₁₃N₃O₄S: 331.35
4-Hydroxy-2-methyl-N-(pyridin-2-yl)-2H-1,1,2-benzothiazine-3-carboxamide 1,1-dioxide [36322-90-0]

Piroxicam contains not less than 98.5% and not more than 101.0% of C₁₅H₁₃N₃O₄S, calculated on the dried basis.

Description Piroxicam occurs as a white to pale yellow crystalline powder.

It is sparingly soluble in acetic anhydride, slightly soluble in acetonitrile, in methanol and in ethanol (99.5), very slightly soluble in acetic acid (100), and practically insoluble in water.

Melting point: about 200°C (with decomposition).

Identification (1) Dissolve 0.1 g of Piroxicam in a mixture of methanol and 0.5 mol/L hydrochloric acid TS (490:1) to make 200 mL. To 1 mL of this solution add the mixture of methanol and 0.5 mol/L hydrochloric acid TS (490:1) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Piroxicam as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve the sample with dichloromethane, evaporate the solvent, dry the residue on a water bath, and perform the test.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Piroxicam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 75 mg of Piroxicam in 50 mL of acetonitrile for liquid chromatography, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile for liquid chromatography to make exactly 10 mL. Pipet 1 mL of this solution, add acetonitrile for liquid chromatography to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than piroxicam obtained with the sample solution is not larger than the peak area of piroxicam with the standard solution, and the total area of the peaks other than piroxicam is not larger than 2 times the peak area of piroxicam with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS, pH 3.0 and acetonitrile for liquid chromatography (3:2).

Flow rate: Adjust the flow rate so that the retention time of piroxicam is about 10 minutes.

Time span of measurement: About 5 times as long as the retention time of piroxicam beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add acetonitrile for liquid chromatography to make exactly 20 mL. Confirm that the peak area of piroxicam obtained with 20 µL of this solution is equivalent to 17.5 to 32.5% of that with 20 µL of the standard solution.

System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of piroxicam are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of piroxicam is not more than 2.0%.

Loss on drying <2.44> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.25 g of Piroxicam, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (1:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 33.14 mg of C₁₅H₁₃N₃O₄S

Containers and storage Containers—Tight containers.
Pivmecillinam Hydrochloride

ピブメシリナム塩酸塩

C₁₉H₃₃N₃O₅S.HCl: 476.03
2,2-Dimethylpropanoyloxyethyl (2S,5R,6R)-6-[azaepan-1-ylmethylene]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrochloride (32887-03-9)

Pivmecillinam Hydrochloride contains not less than 630 μg (potency) and not more than 710 μg (potency) per mg, calculated on the anhydrous basis. The potency of Pivmecillinam Hydrochloride is expressed as mass (potency) of mecillinam (C₁₅H₂₃N₃O₃S: 325.43).

**Description** Pivmecillinam Hydrochloride occurs as a white to yellowish white crystalline powder.

It is very soluble in methanol and in acetic acid (100), freely soluble in water and in ethanol (99.5), and soluble in acetonitrile.

**Identification** (1) Determine the infrared absorption spectrum of Pivmecillinam Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Pivmecillinam Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 0.5 g of Pivmecillinam Hydrochloride in 10 mL of water, and add 1 mL of dilute nitric acid and 1 drop of silver nitrate TS: a white precipitate is formed.

**Optical rotation** <2.49> [α]D: +200 – +220° (1 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

**Purity** (1) Heavy metals <1.07>—To 1.0 g of Pivmecillinam Hydrochloride in a crucible add 0.01 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), fire the ethanol to burn, and heat gradually to incinerate. If a carbonized substance remains, moisten with a small amount of nitric acid, and ignite to incinerate. Cool, add 3 mL of hydrochloric acid to the residue, dissolve by warming on a water bath, and heat to dryness. To the residue add 10 mL of water, and dissolve by warming on a water bath. After cooling, adjust the pH to 3 to 4 with ammonia TS, add 2 mL of dilute acetic acid, filter if necessary, and wash the crucible and the filter with 10 mL of water. Put the filtrate and the washings to a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution in the same manner as the test solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.14>—Prepare the test solution with 1.0 g of Pivmecillinam Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Pivmecillinam Hydrochloride in 4.0 mL of a mixture of acetonitrile and acetic acid (100) (97:3), and use this solution as the sample solution. Separately, dissolve 2.0 mg of Pivmecillinam Hydrochloride RS in 4.0 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.09>. Spot 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography, allow to stand for 30 minutes, then spot 2 μL of the sample solution on the plate. Immediately, develop the plate with a mixture of acetone, water and acetic acid (100) (10:1:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand for 10 minutes in iodine vapor: the spot from the sample solution appeared at the position corresponding to the spot obtained from the standard solution is not larger and not more intense than the spot from the standard solution, and any spot other than the principal spot and the above spot is not observable.

**Water** <2.48> Not more than 1.0% (0.25 g, coulometric titration).

**Assay** Weigh accurately an amount of Pivmecillinam Hydrochloride and Pivmecillinam Hydrochloride RS, equivalent to about 20 mg (potency), dissolve in a suitable amount of the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of pivmecillinam to that of the internal standard.

Amount [μg (potency)] of mecillinam (C₁₅H₂₃N₃O₃S) = M₅ × Q₁/Q₂ × 1000

M₅: Amount [mg (potency)] of Pivmecillinam Hydrochloride RS

**Internal standard solution**—A solution of diphenyl in the mobile phase (1 in 12,500).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.771 g of ammonium acetate in about 900 mL of water, adjust the pH to 3.5 with acetic acid (100), and add water to make 1000 mL. To 400 mL of this solution add 600 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pivmecillinam is about 6.5 minutes.

**System suitability**—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, pivmecillinam and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pivmecillinam to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.
Pivmecillinam Hydrochloride Tablets

ピブメシリナム塩酸塩錠

Pivmecillinam Hydrochloride Tablets contains not less than 93.0% and not more than 107.0% of the labeled potency of mecillinam (C₁₅H₂₃N₃O₃S: 325.43).

**Method of preparation** Prepare as directed under Tablets, with Pivmecillinam Hydrochloride.

**Identification** Powder Pivmecillinam Hydrochloride Tablets, dissolve a portion of the powder, equivalent to 35 mg (potency) of Pivmecillinam Hydrochloride according to the labeled amount, in 4 mL of a mixture of acetonitrile and acetic acid (100) (97:3), and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately dissolve 25 mg of Pivmecillinam Hydrochloride RS in 2 mL of a mixture of acetonitrile and acetic acid (100) (97:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.05>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and immediately develop the plate with a mixture of acetone, water and acetic acid (100) (10:1:1) to a distance of about 12 cm, and air-dry the plate with a mixture of acetone, water and acetic acid (100) (97:3), and filter through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Pivmecillinam Hydrochloride RS, equivalent to about 20 mg (potency), dissolve in the mobile phase, add exactly 10 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Pivmecillinam Hydrochloride.

Amount [mg (potency)] of mecillinam (C₁₅H₂₃N₃O₃S) = \( M_5 \times \frac{Q_1}{Q_2} \times 5 \)

\( M_5 \): Amount [mg (potency)] of Pivmecillinam Hydrochloride RS

**Internal standard solution**—A solution of diphenyl in the mobile phase (1 in 12,500).

**Containers and storage** Containers—Tight containers.

Live Oral Poliomyelitis Vaccine

経口生ポリオワクチン

Live Oral Poliomyelitis Vaccine contains live attenuated poliovirus of type I, II and III.

Monovalent or bivalent product may be prepared, if necessary.

Live Oral Poliomyelitis Vaccine conforms to the requirements of Live Oral Poliomyelitis Vaccine in the Minimum Requirements for Biological Products.

**Description** Live Oral Poliomyelitis Vaccine is a light yellow-red to light red, clear liquid.

Polymixin B Sulfate

ポリミキシン B 硫酸塩

**Disintegration** <6.09> Perform the test using the disk: it meets the requirement.

**Assay** Weigh accurately the mass of not less than 20 Pivmecillinam Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g (potency) of Pivmecillinam Hydrochloride, add 50 mL of the mobile phase, shake vigorously for 10 minutes, and add the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 50 mL, filter through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Pivmecillinam Hydrochloride RS, equivalent to about 20 mg (potency), dissolve in the mobile phase, add exactly 10 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Pivmecillinam Hydrochloride.

\[ \text{Amount [mg (potency)] of mecillinam (C₁₅H₂₃N₃O₃S)} = M_5 \times \frac{Q_1}{Q_2} \times 5 \]

\( M_5 \): Amount [mg (potency)] of Pivmecillinam Hydrochloride RS

\[ \text{Internal standard solution—A solution of diphenyl in the mobile phase (1 in 12,500).} \]

Polymixin B Sulfate is the sulfate of a mixture of peptide substances having antibacterial activity produced by the growth of Bacillus polymyxa.

It contains not less than 6500 units per mg, calculated on the dried basis. The potency of Polymixin B
Sulfate is expressed as mass unit of polymixin B \((C_{55-56}H_{96-98}N_{16}O_{13})\). One unit of Polymixin B Sulfate is equivalent to 0.129 \(\mu\)g of polymixin B sulfate \((C_{55-56}H_{96-98}N_{16}O_{13-2H_2SO_4})\).

**Description** Polymixin B Sulfate occurs as a white to yellow-brown powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

**Identification** (1) To 5 mL of a solution of Polymixin B Sulfate (1 in 10) add 5 mL of a solution of sodium hydroxide (1 in 10), add 5 drops of a solution of copper (II) sulfate pentahydrate (1 in 100) while shaking: a purple color develops.

(2) Transfer 5 mg each of Polymixin B Sulfate and Polymixin B Sulfate RS separately into two glass-stoppered test tubes, add 1 mL of diluted hydrochloric acid (1 in 2), stopper the tube, heat at 135°C for 5 hours, then heat to dryness on a water bath, and keep the heating until no more hydrochloric acid odor is evolved. Dissolve the residue in 0.5 mL of water, and use these solutions as the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4000 units and 1000 units, and use these solutions as the high concentration sample solution, respectively. Then use these solutions as the concentration standard solution and the low concentration sample solution, respectively.

Add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4000 units and 1000 units, and use these solutions as the high concentration sample solution, respectively. Then use these solutions as the concentration standard solution and the low concentration sample solution, respectively.

(3) A solution of Polymixin B Sulfate (1 in 20) responds to the Qualitative Tests <1.09> for sulfate.

**Optical rotation** <2.49> [\(\alpha\)]\(D\)\(\) = -78 – -90° (0.5 g calculated on the dried basis, water, 25 mL, 100 mm).

**pH** <2.54> The pH of a solution obtained by dissolving 1.0 g of Polymixin B Sulfate in 50 mL of water is between 5.0 and 7.0.

**Phenylalanine** Weigh accurately about 0.375 g of Polymixin B Sulfate, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Determine absorbances, \(A_2\), \(A_3\), \(A_4\), \(A_5\) and \(A_6\), of this solution at 252 nm, at 258 nm, at 264 nm, at 280 nm and at 300 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry <2.24>, and calculate the amount of phenylalanine by the following equation: the amount of phenylalanine calculated on the dried basis is not less than 9.0% and not more than 12.0%.

Amount (%) of phenylalanine

\[= \left( A_2 - 0.5A_4 + 0.5A_5 - 1.8A_6 + 0.8A_3 \right)/M_f \times 9.4787 \]

\(M_f\): Amount (g) of the sample, calculated on the dried basis

**Purity** Heavy metals <1.07>—Proceed with 1.0 g of Polymixin B Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** <2.41> Not more than 6.0% (1 g, in vacuum, 60°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.75% (1 g).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Escherichia coli* NIHJ

(ii) Agar media for seed and base layer

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Meat extract</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>30.0 g</td>
</tr>
<tr>
<td>Agar</td>
<td>20.0 g</td>
</tr>
</tbody>
</table>

Water 1000 mL

Mix all the ingredients, and sterilize. Adjust the pH <2.54> of the solution so that it will be 6.5 to 6.6 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Polymixin B Sulfate RS, equivalent to about 200,000 units, dissolve in phosphate buffer solution, pH 6.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 14 days. Take exactly a suitable amount of the standard stock solution before use, and add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4000 units and 1000 units, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Polymixin B Sulfate, equivalent to about 200,000 units, and dissolve in phosphate buffer solution, pH 6.0 to make exactly 20 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4000 units and 1000 units, and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

**Polyoxyl 40 Stearate**

ステアリン酸ポリオキシル 40

Polyoxyl 40 Stearate is the monostearate of condensation polymers of ethylene oxide represented by the formula \(H_2O(CH_2CH_2)_nOCOC_7H_{15}\), in which \(n\) is approximately 40.

**Description** Polyoxyl 40 Stearate occurs as a white to light yellow, waxy solid or powder. It is odorless or has a faint fat-like odor.

It is soluble in water, in ethanol (95) and in diethyl ether.

**Congealing point** <2.42> 39.0 – 44.0°C

**Congealing point of the fatty acid** <1.13> Not below 53°C.

**Acid value** <1.13> Not more than 1.

**Saponification value** <1.13> 25 – 35
Containers and storage
Containers—Tight containers.

Iodine value
\(<1.15\) 19 – 24 Use chloroform instead of cyclohexane, and titrate \(<2.5d\) without using an indicator, until the yellow color of iodine disappears.

Acid value \(<1.12\) Not more than 2.0.

Saponification value \(<1.13\) 45 – 55

Iodine value \(<1.15\) 19 – 24

Purity (1) Heavy metals \(<1.07\)—Proceed with 1.0 g of Polysorbate 80 according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic \(<1.11\)—Prepare the test solution with 1.0 g of Polysorbate 80 according to Method 3, and perform the test (not more than 2 ppm).

Residue on ignition \(<2.44\) Not more than 0.1% (1 g).

Containers and storage Containers—Tight containers.

**Polysorbate 80**

ポリソルベート 80

Polysorbate 80 is a polyoxyethylene ether of anhydrous sorbitol, partially esterified with oleic acid.

**Description** Polysorbate 80 is a colorless or orange-yellow, viscous liquid, having a faint, characteristic odor and a warm, slightly bitter taste.

It is miscible with methanol, with ethanol (95), with warm ethanol (95), with pyridine and with chloroform.

It is freely soluble in water and slightly soluble in diethyl ether. The pH of a solution of Polysorbate 80 (1 in 20) is between 5.5 and 7.5.

**Identification** (1) To 5 mL of a solution of Polysorbate 80 (1 in 20) add 5 mL of sodium hydroxide TS, boil for 5 minutes, cool, and acidify with dilute hydrochloric acid: the solution is opalescent.

(2) To 5 mL of a solution of polysorbate 80 (1 in 20) add 2 to 3 drops of bromine TS: the color of the test solution is discharged.

(3) Mix 6 mL of Polysorbate 80 with 4 mL of water at an ordinary, or lower than ordinary, temperature: a jelly-like mass is produced.

(4) To 10 mL of a solution of Polysorbate 80 (1 in 20) add 5 mL of ammonium thiocyanate-cobalt (II) nitrate TS, shake well, add 5 mL of chloroform, shake, and allow to stand: a blue color develops in the chloroform layer.

**Viscosity** \(<2.53\) 345 – 445 mm²/s (Method 1, 25°C).

**Specific gravity** \(<1.13\) \(d^25_{20}: 1.065 – 1.095\)

**Acid value** \(<1.12\) Not more than 2.0.

**Saponification value** \(<1.13\) 45 – 55

**Iodine value** \(<1.15\) 19 – 24

**Purity** (1) Heavy metals \(<1.07\)—Prepare with 1.0 g of Polysorbate 80 according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic \(<1.11\)—Prepare the test solution with 1.0 g of Polysorbate 80 according to Method 3, and perform the test (not more than 2 ppm).

**Potassium Bromide**

カリ石ケン

Potash Soap contains not less than 40.0% as fatty acids.

**Method of preparation**

Fixed oil 470 mL
Potassium Hydroxide a sufficient quantity
Water, Purified Water or Purified Water in Containers a sufficient quantity

To make 1000 g

Dissolve Potassium Hydroxide, in required quantity for saponification, in Water, Purified Water or Purified Water in Containers, add this solution to fixed oil, previously warmed, add a sufficient quantity of Ethanol if necessary, stir thoroughly, heat in a water bath, and continue the saponification. After complete saponification, add Water, Purified Water or Purified Water in Containers to make 1000 g.

**Description** Potash Soap occurs as a yellow-brown, transparent, unctuous, soft mass, having a characteristic odor.

It is freely soluble in water and in ethanol (95).

**Purity** Silicic acid and alkalinity—Dissolve 10 g of Potash Soap in 30 mL of ethanol (95), and add 0.50 mL of 1 mol/L hydrochloric acid VS: no turbidity is produced. Add 1 drop of phenolphthalein TS to this solution: no red color develops.

**Assay** Weigh accurately about 5 g of Potash Soap, dissolve in 100 mL of hot water, and transfer to a separator. Acidify the mixture with dilute sulfuric acid, and cool. Extract the solution with 50-mL, 40-mL, and 30-mL portions of diethyl ether. Wash the combined diethyl ether extracts with 10-mL portions of water until the washing contains no acid. Transfer the diethyl ether solution to a tared flask, evaporate diethyl ether on a water bath at a temperature as low as possible. Dry the residue at 80°C to constant mass, and weigh as fatty acids.

**Containers and storage** Containers—Tight containers.
white crystals, granules or crystalline powder. It is odorless.

It is freely soluble in water and in glycerin, soluble in hot ethanol (95), and slightly soluble in ethanol (95).

**Identification**  A solution of Potassium Bromide (1 in 10) responds to Qualitative Tests <1.09> for potassium salt and for bromide.

**Purity**  (1) Clarity and color of solution—Dissolve 1.0 g of Potassium Bromide in 3 mL of water: the solution is clear and colorless.

(2) Alkalinity—Dissolve 1.0 g of Potassium Bromide in 10 mL of water, add 0.10 mL of 0.05 mol/L sulfuric acid VS and 1 drop of phenolphthalein TS, heat to boiling, and cool: no color develops.

(3) Chloride—Make a calculation from the result obtained in the Assay: not more than 84.5 mL of 0.1 mol/L silver nitrate VS is consumed for 1 g of Potassium Bromide.

(4) Sulfate <1.14>—Proceed with 2.0 g of Potassium Bromide, and perform the test. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(5) Iodide—Dissolve 0.5 g of Potassium Bromide in 10 mL of water, add 2 to 3 drops of iron (III) chloride TS and 1 mL of chloroform, and shake: no red-purple to purple color develops in the chloroform layer.

(6) Bromate—Dissolve 1.0 g of Potassium Bromide in 10 mL of freshly boiled and cooled water, and add 0.1 mL of potassium iodide TS, 1 mL of starch TS and 3 drops of dilute sulfuric acid. Shake the mixture gently, and allow to stand for 5 minutes: no blue color develops.

(7) Heavy metals <1.07>—Proceed with 2.0 g of Potassium Bromide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(8) Barium—Dissolve 0.5 g of Potassium Bromide in 10 mL of water, add 0.5 mL of dilute hydrochloric acid and 1 mL of potassium sulfate TS, and allow to stand for 10 minutes: no turbidity is produced.

(9) Arsenic <1.11>—Prepare the test solution with 1.0 g of Potassium Bromide according to Method 1, and perform the test (not more than 2 ppm).

**Loss on drying** <2.41>  Not more than 1.0% (1 g, 110°C, 4 hours).

**Assay**  Weigh accurately about 0.4 g of Potassium Bromide, previously dried, and dissolve in 50 mL of water. Add 10 mL of dilute nitric acid and exactly measured 50 mL of 0.1 mol/L silver nitrate VS, and titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS = 11.90 mg of KBr

**Containers and storage**  Containers—Tight containers.

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**Potassium Canrenoate**

Potassium Canrenoate, when dried, contains not less than 98.0% and not more than 102.0% of C_{22}H_{29}KO_{4}.

**Description**  Potassium Canrenoate occurs as a pale yellowish-white to pale yellow-brown, crystalline powder.

It is freely soluble in water, soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in chloroform and in diethyl ether.

**Identification**  (1) Dissolve 2 mg of Potassium Canrenoate in 2 drops of sulfuric acid: an orange color develops. Observe under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence. Add 1 drop of acetic anhydride to this solution: the color of the solution changes to red.

(2) Determine the absorption spectrum of a solution of Potassium Canrenoate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Potassium Canrenoate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.26>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) The solution of Potassium Canrenoate (1 in 10) responds to Qualitative Tests <1.09> (1) for potassium salt.

**Optical rotation** <2.49>  [α]_{D}^{21} = –71 to –76° (after drying, 0.2 g, methanol, 20 mL, 100 mm).

**pH** <2.54>  Dissolve 1.0 g of Potassium Canrenoate in 20 mL of water: the pH of this solution is between 8.4 and 9.4.

**Purity**  (1) Clarity and color of solution—Dissolve 0.5 g of Potassium Canrenoate in 5 mL of water: the solution is clear, and shows a pale yellow to light yellow color.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Potassium Canrenoate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Potassium Canrenoate according to Method 3, and perform the test (not more than 2 ppm).

(4) Canrenone—Place 0.40 g of Potassium Canrenoate in a glass-stoppered centrifuge tube, cool in ice-water to a
temperature not higher than 5°C, add 6 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.0, being cooled to a temperature not higher than 5°C to dissolve, and add 8 mL of water being cooled to a temperature not higher than 5°C. Add exactly 10 mL of chloroform, allow to stand for 3 minutes at a temperature not higher than 5°C, shake vigorously for 2 minutes, and centrifuge. Drain off the water layer, collect 5 mL of the chloroform layer, transfer to a glass-stoppered centrifuge tube containing 3 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.0, cooled to a temperature not higher than 5°C, and 4 mL of water cooled to a temperature not higher than 5°C, shake for 1 minute, and centrifuge. Drain off the water layer, pipet 2 mL of the chloroform layer, and add chloroform to make exactly 10 mL. Determine the absorbance of this solution at 283 nm as directed under Ultraviolet-visible Spectrophotometry. Each mL of 0.1 mol/L perchloric acid VS = 69.11 mg of K₂CO₃.

**Containers and storage**  Containers—Tight containers.

**Potassium Carbonate**

炭酸カリウム

K₂CO₃: 138.21

Potassium Carbonate, when dried, contains not less than 99.0% of K₂CO₃.

**Description**  Potassium Carbonate occurs as white granules or powder. It is odorless.

It is very soluble in water, and practically insoluble in ethanol (95).

A solution of Potassium Carbonate (1 in 10) is alkaline. It is hygroscopic.

**Identification**  A solution of Potassium Carbonate (1 in 10) responds to Qualitative Tests for potassium salt and for carbonate.

**Purity**  (1) Clarity and color of solution—Dissolve 1.0 g of Potassium Carbonate in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals—Dissolve 1.0 g of Potassium Carbonate in 2 mL of water and 6 mL of dilute hydrochloric acid, and evaporate to dryness in a water bath. Dissolve the residue in 35 mL of water and 2 mL of dilute acetic acid, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 6 mL of dilute hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 2.0 mL of Standard Lead Solution to dryness, and dilute with water to 50 mL (not more than 20 ppm).

(3) Sodium—Dissolve 1.0 g of Potassium Carbonate in 20 mL of water, and perform the test as directed under Flame Coloration Test for potassium salt and for chloride.

(4) Arsenic—Prepare the test solution with 0.5 g of Potassium Carbonate, according to Method 1, and perform the test (not more than 4 ppm).

**Loss on drying**  Not more than 0.5% (1 g, 105°C, 4 hours). Assay  Weigh accurately about 0.2 g of Potassium Carnoate, previously dried, dissolve in 75 mL of acetic acid, and titrate with 0.5 mol/L sulfuric acid VS until the blue color of the solution changes to yellow-green, boil cautiously, then cool, and titrate with 0.5 mol/L perchloric acid VS: a yellow-green color develops (indicator: 2 drops of bromocresol green TS).

Each mL of 0.5 mol/L sulfuric acid VS = 69.11 mg of K₂CO₃.

**Potassium Chloride**

塩化カリウム

KCl: 74.55

Potassium Chloride, when dried, contains not less than 99% of KCl.

**Description**  Potassium Chloride occurs as colorless or white crystals or crystalline powder. It is odorless, and has a saline taste.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether. A solution of Potassium Chloride (1 in 10) is neutral.

**Identification**  A solution of Potassium Chloride (1 in 10) responds to Qualitative Tests for potassium salt and for chloride.

**Purity**  (1) Clarity and color of solution—Dissolve 1.0 g of Potassium Chloride in 5 mL of water: the solution is clear and colorless.

(2) Acidity and alkalinity—Dissolve 5.0 g of Potassium Chloride in 50 mL of freshly boiled and cooled water, and add 3 drops of phenolphthalein TS: no red color develops. Then add 0.50 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(3) Bromide—Dissolve 1.0 g of Potassium Chloride in water to make 100 mL. To 5 mL of the solution add 3 drops of dilute hydrochloric acid and 1 mL of chloroform, and add 3 drops of sodium toluidinesulfonchloramide TS dropwise while shaking: no yellow to yellow-red color develops in the chloroform layer.

(4) Iodide—Dissolve 0.5 g of Potassium Chloride in 10 mL of water, add 3 drops of iron (III) chloride TS and 1 mL of chloroform, shake, allow to stand for 30 minutes, and shake again: no red-purple to purple color develops in the chloroform layer.
(5) Heavy metals $<1.07>$—Proceed with 4.0 g of Potassium Chloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 5 ppm).

(6) Calcium and magnesium—Dissolve 0.20 g of Potassium Chloride in 20 mL of water, add 2 mL of ammonia TS, 2 mL of ammonium oxalate TS and 2 mL of disodium hydrogenphosphate TS, and then allow to stand for 5 minutes: no turbidity is produced.

(7) Sodium—Dissolve 1.0 g of Potassium Chloride in 20 mL of water, and perform the Flame Coloration Test $<1.04>$ (1): no persistent, yellow color develops.

(8) Arsenic $<1.17>$—Prepare the test solution with 1.0 g of Potassium Chloride according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying $<2.41>$ Not more than 0.5% (1 g, 130°C, 2 hours).

Assay Weigh accurately about 0.2 g of Potassium Chloride, previously dried, dissolve in 50 mL of water, add 2 mL of ammonium oxalate TS and 2 mL of disodium hydrogenphosphate TS to 4.0 with phosphoric acid. Each mL of 0.1 mol/L silver nitrate VS = 7.455 mg of KCl

Containers and storage Containers—Tight containers.

Potassium Clavulanate クラプラン酸カリウム

$C_9H_8KNO_5$: 237.25
Monopotassium (2R,5R)-3-[[1Z]-2-hydroxyethyldiene]-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate [6177-45-5]

Potassium Clavulanate is the potassium salt of a substance having β-lactamase inhibiting activity produced by the growth of Streptomyces clavuligerus. It contains not less than 810 μg (potency) and not more than 860 μg (potency) per mg, calculated on the anhydrous basis. The potency of Potassium Clavulanate is expressed as mass (potency) of clavulanic acid ($C_9H_8KNO_5$: 199.16).

Description Potassium Clavulanate occurs as a white to light yellowish white, crystalline powder. It is very soluble in water, soluble in methanol, and slightly soluble in ethanol (95). It is hygroscopic.

Identification (1) To 1 mL of a solution of Potassium Clavulanate (1 in 50,000) add 5 mL of imidazole TS, and warm in a water bath at 30°C for 12 minutes. After cooling, determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry $<2.24>$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Potassium Clavulanate as directed in the potassium bromide disk method under Infrared Spectrophotometry $<2.25>$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Potassium Clavulanate responds to Qualitative Tests $<1.09>$ (1) for potassium salt.

Optical rotation $<2.49>$ $[\alpha]_D^20$: +53° to +63° (0.5 g calculated on the anhydrous basis, water, 50 mL, 100 mm).

Purity (1) Heavy metals $<1.07>$—Proceed with 2.0 g of Potassium Clavulanate according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic $<1.17>$—Prepare the test solution with 1.0 g of Potassium Clavulanate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Potassium Clavulanate in 10 mL of the mobile phase A, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography $<2.01>$ according to the following conditions, and determine each peak area by the automatic integration method: the area of each peak other than clavulanic acid from the sample solution is not larger than the peak area of clavulanic acid from the standard solution, and the total area of the peaks other than clavulanic acid from the sample solution is not larger than 2 times the peak area of clavulanic acid from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 230 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase A: Adjust the pH of 0.05 mol/L sodium dihydrogen phosphate TS to 4.0 with phosphoric acid.
Mobile phase B: A mixture of the mobile phase A and methanol (1:1).
Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 4</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>4 – 15</td>
<td>100 → 0</td>
<td>0 → 100</td>
</tr>
<tr>
<td>15 – 25</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Flow rate: 1.0 mL per minute.
Time span of measurement: About 6 times as long as the retention time of clavulanic acid.

System suitability—
Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase A to make exactly 10

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Image 133x387 to 207x428
Potassium Guaiacolsulfonate

ゲアヤコールスルホン酸カリウム

C$_{7}$H$_{7}$K$_{2}$O$_{5}$S: 242.29
Monopotassium 4-hydroxy-3-methoxybenzenesulfonate

\[ \text{C}: \text{H}_{2} \text{NO}_{3}: 242.29 \]

Potassium Guaiacolsulfonate contains not less than 98.5% of C$_{7}$H$_{7}$K$_{2}$O$_{5}$S, calculated on the anhydrous basis.

**Description** Potassium Guaiacolsulfonate occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor and a slightly bitter taste.

It is freely soluble in water and in formic acid, soluble in methanol, and practically insoluble in ethanol (95), in acetic anhydride and in diethyl ether.

**Identification** (1) To 10 mL of a solution of Potassium Guaiacolsulfonate (1 in 100) add 2 drops of iron (III) chloride TS: a blue-purple color develops.

(2) Dissolve 0.25 g of Potassium Guaiacolsulfonate in water to make 500 mL, and to 10 mL of this solution add phosphate buffer solution, pH 7.0, to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.10>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Potassium Guaiacolsulfonate (1 in 10) responds to Qualitative Tests <1.09> for potassium salt.

**pH** <2.54> Dissolve 1.0 g of Potassium Guaiacolsulfonate in 20 mL of water: the pH of the solution is between 4.0 and 5.5.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Potassium Guaiacolsulfonate in 20 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 0.8 g of Potassium Guaiacolsulfonate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.03%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Potassium Guaiacolsulfonate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.17>—Prepare the test solution with 1.0 g of Potassium Guaiacolsulfonate according to Method 1, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.20 g of Potassium Guaiacolsulfonate in 200 mL of mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.0> according to the following conditions. Determine each peak
area obtained from these solutions by the automatic integration method: the total area of peaks other than the peak of potassium guaiacolsulfonate from the sample solution is not larger than the peak area of potassium guaiacolsulfonate from the standard solution.

**Operating conditions**—
- Detector: An ultraviolet absorption photometer (wavelength: 279 nm).
- Column: A stainless steel column 4 mm in inside diameter and 20 to 25 cm in length, packed with dimethyldimethylamino-propylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).
- Column temperature: A constant temperature of about 30°C.
- Mobile phase: A mixture of 0.05 mol/L potassium dihydrogenphosphate TS and methanol (20:1).
- Flow rate: Adjust the flow rate so that the retention time of potassium guaiacolsulfonate is about 10 minutes.
- Selection of column: Weigh 50 mg each of potassium guaiacolsulfonate and guaiacol, and dissolve in 50 mL of the mobile phase. Proceed with 5 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of guaiacol and potassium guaiacolsulfonate in this order with the resolution of these peaks being not less than 4.
- Detection sensitivity: Adjust the sensitivity so that the peak height of potassium guaiacolsulfonate from the sample solution is not larger than the peak area of potassium guaiacolsulfonate from the standard solution.
- Time span of measurement: About twice as long as the retention time of potassium guaiacolsulfonate.

**Water** <2. 48> 3.0 – 4.5% (0.3 g, volumetric titration, direct titration).

**Assay** Weigh accurately about 0.3 g of Potassium Guaiacolsulfonate, dissolve in 2.0 mL of formic acid, add 50 mL of acetic anhydride, and titrate <2. 50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 24.23 mg of C7H7KO5S

**Containers and storage** Containers—Well-closed containers.
- Storage—Light-resistant.

**Potassium Hydroxide**

水酸化カリウム

KOH: 56.11

Potassium Hydroxide contains not less than 85.0% of KOH.

**Description** Potassium Hydroxide occurs as white fused masses, in small pellets, in flakes, in sticks and in other forms. It is hard and brittle, and shows a crystalline fracture.
- It is freely soluble in water and in ethanol (95), and practically insoluble in diethyl ether.
- It rapidly absorbs carbon dioxide in air.
- It deliquesces in the presence of moisture.

**Identification** (1) A solution of Potassium Hydroxide (1 in 500) is alkaline.

(2) A solution of Potassium Hydroxide (1 in 25) responds to Qualitative Tests <1. 09> for potassium salt.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Potassium Hydroxide in 20 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 2.0 g of Potassium Hydroxide in water, and add water to make 100 mL. To 25 mL of the solution add 8 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.7 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.050%).

(3) Heavy metals <1.07>—Dissolve 1.0 g of Potassium Hydroxide in 5 mL of water, add 7 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, 2 mL of dilute acetic acid and 1 drop of ammonia TS, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 7 mL of dilute hydrochloric acid on a water bath to dryness, dissolve the residue in 2 mL of dilute acetic acid and 3.0 mL of Standard Lead Solution, and add water to make 50 mL (not more than 30 ppm).

(4) Sodium—Dissolve 0.10 g of Potassium Hydroxide in 10 mL of dilute hydrochloric acid, and perform the test as directed under Flame Coloration Test <1.04> (1): no persistent yellow color develops.

(5) Potassium carbonate—The amount of potassium carbonate (K2CO3: 138.21) is not more than 2.0% when calculated by the following equation using B (mL) obtained in the Assay.

Amount of potassium carbonate (mg) = 138.21 × B

**Assay** Weigh accurately about 1.5 g of Potassium Hydroxide, and dissolve in 40 mL of freshly boiled and cooled water. Cool the solution to 15°C, add 2 drops of phenolphthalein TS, and titrate <2. 50> with 0.5 mol/L sulfuric acid VS until the red color of the solution disappears. Record the amount A (mL) of 0.5 mol/L sulfuric acid VS consumed, then add 2 drops of methyl orange TS, and titrate <2. 50> again with 0.5 mol/L sulfuric acid VS until the solution changes to a persistent light red color. Record the amount B (mL) of 0.5 mol/L sulfuric acid VS consumed.

Calculate the amount KOH from the amount, A (mL) – B (mL).

Each mL of 0.5 mol/L sulfuric acid VS = 56.11 mg of KOH

**Containers and storage** Containers—Tight containers.

**Potassium Iodide**

ヨウ化カリウム

KI: 166.00

Potassium Iodide, when dried, contains not less than 99.0% of KI.

**Description** Potassium Iodide occurs as colorless or white
crystals, or a white crystalline powder.
   It is very soluble in water, soluble in ethanol (95), and
   practically insoluble in diethyl ether.
   It is slightly deliquescent in moist air.

Identification
A solution of Potassium Iodide (1 in 20) responds to Qualitative Tests <1.09> for potassium salt and for iodide.

Purity
(1) Clarity and color of solution—Dissolve 1.0 g of Potassium Iodide in 2 mL of water: the solution is clear and colorless.
   (2) Alkalinity—Dissolve 1.0 g of Potassium Iodide in 10 mL of freshly boiled and cooled water, and add 0.50 mL of 0.005 mol/L sulfuric acid VS and 1 drop of phenolphthalein TS: no color develops.
   (3) Chloride, bromide and thiosulfate—Dissolve 0.20 g of Potassium Iodide in 5 mL of ammonia TS, add 15.0 mL of 0.1 mol/L silver nitrate VS, shake for 2 to 3 minutes, and filter. To 10 mL of the filtrate, add 15 mL of dilute nitric acid: no brown color develops. The solution has no more turbidity than that of the following control solution.
   Control solution: To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 2.5 mL of ammonia TS, and 2 mL of sodium hydroxide TS and 0.2 g of aluminum wire. Insert the absorbent cotton in the mouth of the test tube, and place a piece of moistened red litmus paper on it. Heat the test tube carefully on a water bath for 15 minutes: the gas evolved does not turn red litmus paper to blue.
   (4) Nitrate, nitrite and ammonium—Place 1.0 g of Potassium Iodide in a 40-mL test tube, and add 5 mL of water, 5 mL of sodium hydroxide TS and 0.2 g of aluminum wire. Warm the mixture to a temperature between 55°C and 60°C, then allow the flask to stand until the red color disappears.
   (5) Cyanide—Dissolve 0.5 g of Potassium Iodide in 10 mL of water. To 5 mL of this solution add 1 drop of iron (II) sulfate TS and 2 mL of sodium hydroxide TS, warm, then add 4 mL of hydrochloric acid: no green color develops.
   (6) Iodate—Dissolve 0.5 g of Potassium Iodide in 10 mL of freshly boiled and cooled water, and add 2 drops of dilute sulfuric acid and 1 drop of starch TS: no blue color develops immediately.
   (7) Heavy metals <1.07>—Proceed with 2.0 g of Potassium Iodide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).
   (8) Barium—Dissolve 0.5 g of Potassium Iodide in 10 mL of water, add 1 mL of dilute sulfuric acid, and allow to stand for 5 minutes: no turbidity is produced.
   (9) Sodium—Dissolve 1.0 g of Potassium Iodide in 10 mL of water, and perform the Flame Coloration Test (1) <1.09>: a yellow color develops, but does not persist.
   (10) Arsenic <1.11>—Prepare the test solution with 0.40 g of Potassium Iodide according to Method 1, and perform the test (not more than 5 ppm).

Loss on drying <2.41> Not more than 1.0% (2 g, 105°C, 4 hours).

Assay
Weigh accurately about 0.5 g of Potassium Iodide, previously dried, in an iodine flask, dissolve in 10 mL of water, add 35 mL of hydrochloric acid and 5 mL of chloroform, and titrate <2.50> with 0.05 mol/L potassium iodate VS with shaking until the red-purple color of the chloroform layer disappears. The end point is reached when the red-purple color does not reappear in the chloroform layer within 5 minutes after the layer has been decolorized.

Each mL of 0.05 mol/L potassium iodate VS = 16.60 mg of KI

Containers and storage
Containers—Tight containers.

Containers—Tight containers.

Storage—Light-resistant.

Potassium Permanganate
通マンガン酸カリウム

KMnO₄: 158.03

Potassium Permanganate, when dried, contains not less than 99.0% of KMnO₄.

Description
Potassium Permanganate occurs as dark purple crystals and has a metallic luster.

It is soluble in water.

A solution of Potassium Permanganate (1 in 1000) has a slightly sweet, astringent taste.

Identification
A solution of Potassium Permanganate (1 in 1000) responds to Qualitative Tests <1.09> for permanganate.

Purity
(1) Water-insoluble substances—Dissolve 2.0 g of Potassium Permanganate, previously powdered, in 200 mL of water. Filter the insoluble substances through a tared filter. To 10 mL of the filtrate, add 1 mL of sulfuric acid, and continue the titration with potassium iodate VS with shaking until the red-purple color of the chloroform layer disappears. The end point is reached when the red-purple color does not reappear in the chloroform layer within 5 minutes after the layer has been decolorized.

Each mL of 0.05 mol/L potassium iodate VS
= 16.60 mg of KMnO₄

Containers and storage
Containers—Tight containers.

Storage—Light-resistant.

Containers—Tight containers.
Potassium Sulfate

**Description** Potassium Sulfate occurs as colorless crystals or a white, crystalline powder. It has a slightly saline, somewhat bitter taste. It is soluble in water and practically insoluble in ethanol (95%).

**Identification** A solution of Potassium Sulfate (1 in 20) responds to Qualitative Tests <1.09> for potassium salt and for sulfate.

**Purity** (1) Clarity and color of solution, and acid or alkali—Dissolve 1.0 g of Potassium Sulfate in 20 mL of water; the solution is clear, colorless and neutral.

(2) Chloride <1.05>—Perform the test with 0.5 g of Potassium Sulfate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Potassium Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Sodium—Dissolve 1.0 g of Potassium Sulfate in 20 mL of water, and perform the test as directed under Flame Coloration Test <1.04> (1); no persistent yellow color develops.

(5) Arsenic <1.11>—Prepare the test solution with 0.40 g of Potassium Sulfate according to Method 1, and perform the test (not more than 5 ppm).

**Loss on drying <2.41>** Not more than 1.0% (1 g, 110°C, 4 hours).

**Assay** Weigh accurately about 0.5 g of Potassium Sulfate, previously dried, boil with 200 mL of water and 1.0 mL of hydrochloric acid, and add gradually 8 mL of boiling barium chloride TS. Heat the mixture on a water bath for 1 hour, collect the precipitate, and wash the precipitate with water until the last washing shows no opalescence on the addition of silver nitrate TS. Dry, heat strongly to constant mass until the last washing shows no opalescence on the addition of silver nitrate TS. Collect the precipitate, and wash the precipitate with water.

Heating on a water bath for 1 hour.

**Collect the precipitate, and wash the precipitate with water.**

**chloride TS.** Heat the mixture on a water bath for 1 hour, hydrochloric acid, and add gradually 8 mL of boiling barium

**Purity (1)**

- **Clarity and color of solution, and acid or alkali**—Dissolve 1.0 g of Potassium Sulfate in 20 mL of water; the solution is clear, colorless and neutral.
- **Chloride** <1.05>—Perform the test with 0.5 g of Potassium Sulfate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).
- **Heavy metals** <1.07>—Proceed with 2.0 g of Potassium Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).
- **Sodium**—Dissolve 1.0 g of Potassium Sulfate in 20 mL of water, and perform the test as directed under Flame Coloration Test <1.04> (1); no persistent yellow color develops.
- **Arsenic** <1.11>—Prepare the test solution with 0.40 g of Potassium Sulfate according to Method 1, and perform the test (not more than 5 ppm).
- **Loss on drying** <2.41> Not more than 1.0% (1 g, 110°C, 4 hours).

**Assay** Weigh accurately about 0.5 g of Potassium Sulfate, previously dried, boil with 200 mL of water and 1.0 mL of hydrochloric acid, and add gradually 8 mL of boiling barium chloride TS. Heat the mixture on a water bath for 1 hour, collect the precipitate, and wash the precipitate with water until the last washing shows no opalescence on the addition of silver nitrate TS. Dry, heat strongly to constant mass between 500°C and 600°C by raising the temperature gradually, and weigh as barium sulfate (BaSO4: 233.39).

- **Amount (mg) of K2SO4**
  - \( \text{amount (mg) of barium sulfate (BaSO}_4\text{)} \times 0.747 \)

**Containers and storage** Containers—Well-closed containers.

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Potato Starch

**Description** Potato Starch occurs as a white powder.

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the test that are not harmonized are marked with symbols (● ●).

**Identification** (1) Examined under a microscope <5.01> using a mixture of water and glycerin (1:1), Potato Starch presents granules, either irregularly shaped, ovoid or pear-shaped, usually 30 – 100 µm in size but occasionally exceeding 100 µm, or rounded, 10 – 35 µm in size. There are occasional compound granules having two to four components. The ovoid and pear-shaped granules have an eccentric hilum and the rounded granules acentric or slightly eccentric hilum. All granules show clearly visible concentric striations. Between orthogonally oriented polarizing plates or prisms, the granules show a distinct black cross intersecting at the hilum.

(2) To 1 g of Potato Starch add 50 mL of water, boil for 1 minute, and allow to cool: a subtle white-turbid, pasty liquid is formed.

(3) To 1 mL of the pasty liquid obtained in (2) add 0.05 mL of diluted iodine TS (1 in 10): an orange-red to deep blue color is formed, and the color disappears by heating.

**pH** <2.54> Put 5.0 g of Potato Starch in a non-metal vessel, add 25.0 mL of freshly boiled and cooled water, mix gently for 1 minute, and allow to stand for 15 minutes: the pH of the solution is between 5.0 and 8.0.

**Purity** (1) Iron—To 1.5 g of Potato Starch add 15 mL of 2 mol/L hydrochloric acid TS, mix, filter, and use the filtrate as the test solution. To 2.0 mL of Standard Iron Solution add water to make 20 mL, and use as the control solution. Put 10 mL each of the test solution and the control solution in test tubes, add 2 mL of a solution of citric acid (1 in 5) and 0.1 mL of mercapto acetic acid, and mix. Alkalize with ammonia solution (28) to litmus paper, add water to make 20 mL, and mix. Transfer 10 mL each of these solutions into test tubes, allow to stand for 5 minutes, and compare the color of these solutions against a white background: the color of the test solution is not darker than that of the control solution (not more than 10 ppm).

(2) Oxidizing substances—To 4.0 g of Potato Starch add 50.0 mL of water, shake for 5 minutes, and centrifuge. To 30.0 mL of the supernatant liquid add 1 mL of acetic acid (100) and 0.5 to 1.0 g of potassium iodide, shake, and allow to stand for 25 to 30 minutes at a dark place. Add 1 mL of starch TS, and titrate <2.59> with 0.002 mol/L sodium thiosulfate VS until the color of the solution disappears. Perform a blank determination and make any necessary correction: the volume of 0.002 mol/L sodium thiosulfate VS consumed is not more than 1.4 mL (not more than 20 ppm,
(3) Sulfur dioxide—
   (i) Apparatus Use as shown in the figure.

   A: Boiling flask (500 mL)
   B: Funnel (100 mL)
   C: Condenser
   D: Test-tube
   E: Tap

   (ii) Procedure Introduce 150 mL of water into the boiling flask, close the tap of the funnel, and pass carbon dioxide through the whole system at a rate of 100 ± 5 mL per minute. Pass cooling water through the condenser, and place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test-tube. After 15 minutes, remove the funnel without interrupting the stream of carbon dioxide, and introduce through the opening into the flask about 25 g of Potato Starch, accurately weighed, with the aid of 100 mL of water. Apply tap grease to the outside of the connection part of the funnel, and load the funnel. Close the tap of the funnel, pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the tap to introduce the hydrochloric acid into the flask, and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide. Place the flask in a water bath, and heat the mixture for 1 hour. Transfer the contents of the test-tube with the aid of a little water to a wide-necked conical flask. Heat in a water bath for 15 minutes, and cool. Add 0.1 mL of bromophenol blue TS, and titrate until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination and make any necessary correction. Calculate the amount of sulfur dioxide by applying the following formula: it is not more than 50 ppm.

   Amount (ppm) of sulfur dioxide = \( \frac{V}{M} \times 1000 \times 3.203 \)
   
   M: Amount (g) of the sample
   V: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

   *4 Foreign matter—Under a microscope \(<5.01>\), Potato Starch does not contain starch granules of any other origin. It may contain a minute quantity, if any, of fragments of the tissue of the original plant.\end{itemize}

| JP XVI | Official Monographs / Povidone | 1273 |

**Povidone**

**Polyvidone**

**Polyvinylpyrrolidone**

\((C_6H_9NO)_n\)

Poly[(2-oxopyrrolidin-1-yl)ethylene]

[9003-39-8]

Povidone is a chain polymer of 1-vinyl-2-pyrrolidone.

It contains not less than 11.5% and not more than 12.8% of nitrogen (N: 14.01), calculated on the anhydrous basis.

It has a nominal K-value of not less than 25 and not more than 90.

The nominal K-value is shown on the label.

**Description** Povidone occurs as a white to slightly yellowish fine powder. It is odorless or has a faint, characteristic odor.

It is freely soluble in water, in methanol and in ethanol (95), slightly soluble in acetone, and practically insoluble in diethyl ether.

It is hygroscopic.

**Identification** Determine the infrared absorption spectrum of Povidone, previously dried at 105°C for 6 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry \(<2.25>\), and compare the spectrum with the Reference Spectrum or the spectrum of Povidone RS previously dried at 105°C for 6 hours: both spectra exhibit similar intensities of absorption at the same wave numbers.

**pH** \(<2.54>\) Dissolve 1.0 g of Povidone in 20 mL of water: the pH of this solution is between 3.0 and 5.0 for Povidone having the nominal K-value of 30 or less, and between 4.0 and 7.0 for Povidone having the nominal K-value exceeding 30.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Povidone in 20 mL of water: the solution is clear and colorless to pale yellow, or pale red.

(2) Heavy metals \(<1.07>—\) Proceed with 2.0 g of Povidone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Aldehydes—Weigh accurately about 1.0 g of Povi-
done and dissolve in 0.05 mol/L pyrophosphate buffer solution, pH 9.0 to make exactly 100 mL. Stopper, heat at 60°C for 60 minutes, allow to cool to room temperature, and use this solution as the sample solution. Separately, dissolve 0.100 g of freshly distilled acetaldehyde in water previously cooled to 4°C to make exactly 100 mL. Allow to stand at 4°C for about 20 hours, pipet 1 mL of this solution, add 0.05 mol/L pyrophosphate buffer solution, pH 9.0 to make exactly 100 mL, and use this solution as the standard solution. Measure 0.5 mL of each of the sample solution, standard solution and water (for blank test), transfer to separate cells, add 2.5 mL of 0.05 mol/L pyrophosphate buffer solution, pH 9.0, and 0.2 mL of β-nicotinamide adenine dinucleotide TS to each of these cells, mix and stopper tightly. Allow to stand for 30 minutes, and perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as the control solution. Determine the absorbances, \( A_{T1}, A_{S1} \) and \( A_{B1} \) of the subsequent solutions of the sample solution, the standard solution and water at 340 nm. Add 0.05 mL of aldehyde dehydrogenase solution to each of the cells, mix and stopper tightly. Allow to stand for 5 minutes at 22 ± 2°C. Determine the absorbances, \( A_{T2}, A_{S2} \) and \( A_{B2} \) of these solutions in the same manner as above: the content of aldehydes is not more than 500 ppm (expressed as acetaldehyde).

\[
\text{Content (ppm) of aldehydes expressed as acetaldehyde} = \frac{1000 \times (A_{T2} - A_{T1} - A_{B2} - A_{B1})}{M} \times (A_{S2} - A_{S1}) - (A_{B2} - A_{B1})
\]

\( M: \) Amount (g) of Povidone, calculated on the anhydrous basis

(4) 1-Vinyl-2-pyrrolidone—Weigh accurately about 0.25 g of Povidone, dissolve in diluted methanol (1 in 5) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 50 mg of 1-vinyl-2-pyrrolidone in methanol to make exactly 100 mL. Pipet 1 mL of this solution and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of 1-vinyl-2-pyrrolidone in each solution: the content of 1-vinyl-2-pyrrolidone is not more than 10 ppm.

\[
\text{Content (ppm) of 1-vinyl-2-pyrrolidone} = 2.5/M \times A_T/A_S
\]

\( M: \) Amount (g) of Povidone, calculated on the anhydrous basis

**Operating conditions—**

Detector: An ultraviolet spectrophotometer (detection wavelength: 254 nm).

Column: Stainless steel columns about 4 mm in inside diameter and about 25 mm in length, and about 4 mm in inside diameter and about 250 mm in length, packed with octyl-silanized silica gel for liquid chromatography (5 μm in particle diameter), and use them as a guard column and a separation column, respectively.

Column temperature: A constant temperature of about 40°C.

**Mobile phase:** A mixture of water and methanol (4:1).

Flow rate: Adjust the flow rate so that the retention time of 1-vinyl-2-pyrrolidone is about 10 minutes.

Selection of column: Dissolve 0.01 g of 1-vinyl-2-pyrrolidone and 0.5 g of vinyl acetate in 100 mL of methanol. To 1 mL of this solution add diluted methanol (1 in 5) to make 100 mL. Proceed with 50 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of 1-vinyl-2-pyrrolidone and vinyl acetate in this order with the resolution between these peaks being not less than 2.0.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of 1-vinyl-2-pyrrolidone obtained from 50 μL of the standard solution is between 10 mm and 15 mm.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of obtained peak areas of 1-vinyl-2-pyrrolidone is not more than 2%.

Washing of the guard column: After each test with the sample solution, wash away the polymeric material of Povidone from the guard column by passing the mobile phase through the column backwards for about 30 minutes at the same flow rate as applied in the test.

(5) Peroxides—Weigh exactly an amount of Povidone, equivalent to 4.0 g calculated on the anhydrous basis, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. To 25 mL of the sample solution add 2 mL of titanium (III) chloride-sulfuric acid TS, and mix. Allow to stand for 30 minutes, and perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared by adding 2 mL of 13% sulfuric acid to 25 mL of the sample solution as a blank: the absorbance of the subsequent solution of the sample solution at 405 nm is not more than 0.35 (not more than 400 ppm, expressed as hydrogen peroxide).

(6) Hydrazine—Transfer 2.5 g of Povidone to a 50-mL centrifuge tube, add 25 mL of water, and stir to dissolve. Add 500 μL of a solution of salicylaldehyde in methanol (1 in 20), stir and warm at 60°C for 15 minutes in a water bath. Allow to cool, add 2.0 mL of toluene, stopper tightly, shake vigorously for 2 minutes, centrifuge, and use the upper layer of the mixture as the sample solution. Separately, dissolve 0.09 g of salicylaldehyde in toluene to make exactly 100 mL. Pipet 1 mL of this solution, add toluene to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 10 μL each of the sample solution and standard solution on a plate coated with a 0.25-mm layer of dimethylsilanized silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol and water (2:1) to a distance of about three-fourths of the length of the plate, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the RI value of the fluorescent spot from the standard solution is about 0.3, and the fluorescence of the spot from the sample solution corresponding to the spot from the standard solution is not more intense than that of the spot from the standard solution (not more than 1 ppm).

**Water** <2.48> Not more than 5.0% (0.5 g, volumetric titration, direct titration).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).
K-value  Weigh accurately an amount of Povidone, equivalent to 1.00 g calculated on the anhydrous basis, and dissolve in water to make exactly 100 mL, allow to stand for 60 minutes, and use this solution as the sample solution. Perform the test with the sample solution and with water at 25°C as directed in Method I under Viscosity Determination <2.53>, and calculate the K-value by the following formula.

\[ K = \frac{1.5 \log \eta_{\text{rel}} - 1}{0.15 + 0.003 c} + \frac{200 c \log \eta_{\text{rel}} + (c + 1.5 c \log \eta_{\text{rel}})^2}{0.15 c + 0.003 c^2} \]

\( c \): Mass (g) of Povidone in 100 mL of the solution, calculated on the anhydrous basis
\( \eta_{\text{rel}} \): Kinematic viscosity of the sample solution relative to that of water

The K-value of Povidone is not less than 90% and not more than 108% of the nominal K-value.

Assay  Weigh accurately about 0.1 g of Povidone, and place in a Kjeldahl flask. Add 5 g of a powdered mixture of 33 g of potassium sulfate, 1 g of copper (II) sulfate pentahydrate and 1 g of titanium (IV) oxide, and wash down any adhering sample from the neck of the flask with a small amount of water. Add 7 mL of sulfuric acid allowing to flow down the inside wall of the flask. Heat the flask gradually over a free flame until the solution has a clear, yellow-green color and the inside wall of the flask is free from a carboxamidaceous material, and then heat for further 45 minutes. After cooling, add cautiously 20 mL of water, cool the solution, and connect the flask to the distillation apparatus previously washed by passing steam through it. To the absorption flask add 30 mL of a solution of boric acid (1 in 25), 3 drops of bromocresol green-methyl red TS and sufficient mol/L hydrochloric acid TS until the color of iodine completely disappears. To this solution add exactly 25 mL of 0.1 mol/L ammonium thiocyanate-cobalt (II) nitrate TS and 2 drops of 1 mL of hydrochloric acid TS: a blue color develops, and a blue precipitate is gradually formed.

Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.025 mol/L sulfuric acid VS = 0.700 mg of N

Containers and storage  Containers—Tight containers.

### Povidone-Iodine

ポビドンヨード

Povidone-Iodine is a complex of iodine with 1-vinyl-2-pyrrolidone polymer.

It contains not less than 9.0% and not more than 12.0% of available iodine (I: 126.90), and not less than 9.5% and not more than 11.5% of nitrogen (N: 14.01), calculated on the dried basis.

**Description**  Povidone-Iodine occurs as a dark red-brown powder. It has a faint, characteristic odor.

It is freely soluble in water and in ethanol (99.5).

The pH of a solution obtained by dissolving 1.0 g of Povidone-Iodine in 100 mL of water is between 1.5 and 3.5.

**Identification (1)**  To 10 mL of diluted starch TS (1 in 10) add 1 drop of a solution of Povidone-Iodine (1 in 10): a deep blue color develops.

(2)  To 1 mL of a solution of Povidone-Iodine (1 in 100) add 1 mL of sodium thiocyanate-cobalt (II) nitrate TS and 2 drops of 1 mol/L hydrochloric acid TS: a blue color develops, and a blue precipitate is gradually formed.

**Purity (1)**  Clarity and color of solution—Dissolve 0.30 g of Povidone-Iodine in 100 mL of water: the solution is clear and brown.

(2)  Heavy metals <1.0>—Proceed with 1.0 g of Povidone-Iodine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3)  Arsenic <1.1>—Prepare the test solution with 1.0 g of Povidone-Iodine according to Method 4, and perform the test (not more than 2 ppm).

(4)  Iodide ion—Weigh accurately about 0.5 g of Povidone-Iodine, dissolve in 100 mL of water, and add sodium hydrosulfite TS until the color of iodine completely disappears. To this solution add exactly 25 mL of 0.1 mol/L silver nitrate VS, shake well with 10 mL of nitric acid, titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS until the solution develops a red-brown color, and calculate the total amount of iodine (indicator: 1 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L ammonium thiocyanate VS = 12.69 mg of I

Obtain the amount of iodide ion, calculated on the dried basis, by deducting the amount (%: of available iodine from the total amount (%) of iodine: it is not more than 6.6%.

**Loss on drying <2.41>**  Not more than 8.0% (1 g, 100°C, 3 hours).
Residue on ignition <2.44>  Not more than 0.05% (5 g).

Assay (1) Available iodine—Weigh accurately about 0.5 g of Povidone-Iodine, dissolve in 30 mL of water, and titrate <2.50> with 0.02 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS).

Each mL of 0.02 mol/L sodium thiosulfate VS = 2.538 mg of I

(2) Nitrogen—Weigh accurately about 20 mg of Povidone-Iodine, and perform the test as directed under Nitrogen Determination <1.085>.

Containers and storage  Containers—Tight containers.

Pranoprofen

プラノプロフェン

C_{15}H_{13}NO_{3}: 255.27

(2RS)-2-(10H-9-Oxa-1-azaanthracen-6-yl)propanoic acid

[52549-17-4]

Pranoprofen, when dried, contains not less than 98.5% of C_{15}H_{13}NO_{3}.

Description  Pranoprofen occurs as a white to pale yellowish-white crystalline powder.

It is freely soluble in N,N-dimethylformamide, soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in acetonitrile, in ethanol (95) and in acetic anhydride, very slightly soluble in diethyl ether, and practically insoluble in water.

A solution of Pranoprofen in N,N-dimethylformamide (1 in 30) shows no optical rotation.

Identification (1) Dissolve 0.02 g of Pranoprofen in 1 mol/L hydrochloric acid TS to make 100 mL, and dilute 10 mL of the solution with water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pranoprofen as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60>  186–190°C

Purity (1) Chloride <1.03>—Dissolve 0.5 g of Pranoprofen in 40 mL of methanol, and 6 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows. To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 40 mL of methanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%)

(2) Heavy metals <1.07>—Proceed with 2.0 g of Pranoprofen according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of the Standard Lead Solution (not more than 10 ppm).

(3) Related Substances—Dissolve 50 mg of Pranoprofen in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area from both solutions by the automatic integration method: the each area of the peaks other than the peak of pranoprofen from the sample solution is not larger than the peak area of pranoprofen from the standard solution, and the total peak area of them is not larger than 2 times the peak area of pranoprofen from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column about 6 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 7.02 g of sodium perchlorate monohydrate in 1000 mL of water, and adjust the pH to 2.5 with perchloric acid. To 2 volumes of this solution add 1 volume of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pranoprofen is about 10 minutes.

Selection of column: Dissolve 4 mg each of Pranoprofen and ethyl parahydroxybenzoate in 200 mL of the mobile phase. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of pranoprofen and ethyl parahydroxybenzoate in this order with the resolution between these peaks being not less than 2.1.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of pranoprofen from 10 μL of the standard solution is between 10 mm and 20 mm.

Time span of measurement: About three times as long as the retention time of pranoprofen.

Loss on drying <2.41>  Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44>  Not more than 0.1% (1 g).

Assay  Weigh accurately about 0.4 g of Pranoprofen, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 25.53 mg of C_{15}H_{13}NO_{3}

Containers and storage  Containers—Tight containers.

Storage—Light-resistant.
Pravastatin Sodium

プラバスタチンナトリウム

C₂₉H₃₅NaO₇·4·4·6·5·1
Monosodium (3R,5R)-3,5-dihydroxy-7-[(1S,2S,6S,8S,8aR)-6-hydroxy-2-methyl-8-[(2S)-2-methylbutanoyloxy]-1,2,6,7,8,8a-hexahydrophtalene-1-yl]heptanoate [81131-70-6]

Pravastatin Sodium contains not less than 98.5% and not more than 101.0% of C₂₉H₃₅NaO₇, calculated on the anhydrous basis and corrected on the amount of the residual solvent.

Description Pravastatin Sodium occurs as a white to yelowish white, powder or crystalline powder.

It is freely soluble in water and in methanol, and soluble in ethanol (99.5).

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Pravastatin Sodium (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pravastatin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25: it exhibits absorption at the wave numbers of about 2970 cm⁻¹, 2880 cm⁻¹, 1727 cm⁻¹ and 1578 cm⁻¹.

(3) Dissolve 50 mg of Pravastatin Sodium in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 24 mg of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.06. Spot 2 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and acetic acid (100:80:16:1) to a distance of about 8 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm); the color tone and the Rf value of the principal spot with the sample solution are not different with them of the spot with the standard solution.

(4) A solution of Pravastatin Sodium (1 in 10) responds to Qualitative Tests 1.09 (1) for sodium salt.

Optical rotation 2.49: +153° to +159° (0.1 g calculated on the anhydrous basis and corrected on the amount of residual solvent, water, 20 mL, 100 mm).

pH 2.5: The pH of a solution obtained by dissolving 1.0 g of Pravastatin Sodium in 20 mL of freshly boiled and cooled water is between 7.2 and 8.2.

Purity (1) Heavy metals 1.07 — Proceed with 1.0 g of Pravastatin Sodium according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.10 g of Pravastatin Sodium in 100 mL of a mixture of water and methanol (11:9), and use this solution as the sample solution. Pipet 10 mL of the sample solution, add the mixture of water and methanol (11:9) to make exactly 100 mL. Pipet 5 mL of this solution, add the mixture of water and methanol (11:9) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography 2.07 according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than pravastatin is not larger than 1/5 times the peak area of pravastatin from the standard solution, and the total area of the peaks other than pravastatin is not larger than the peak area of pravastatin from the standard solution. Keep the sample solution and standard solution at not over than 15°C.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of pravastatin beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add a mixture of water and methanol (11:9) to make exactly 50 mL. Confirm that the peak area of pravastatin obtained with 10 µL of this solution is equivalent to 7 to 13% of that with 10 µL of the standard solution.

System performance: Dissolve 5 mg of pravastatin sodium in 50 mL of the mixture of water and methanol (11:9). When the procedure is run with 10 µL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pravastatin are not less than 3500 and not more than 1.6, respectively.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pravastatin is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Water 2.48: Not more than 4.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.1 g of Pravastatin Sodium, and dissolve in a mixture of water and methanol (11:9) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the mixture of water and methanol (11:9) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS (previously determine the water 2.48 with 0.5 g by direct titration in volumetric titration) dissolve in the mixture of water and methanol (11:9) to make exactly 25 mL. Proceed with exactly 10 mL of this solution in the same manner for the preparation of the sample solution, and use the solution so obtained as the standard solution. Per-
form the test with 10 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_3 \), of the peak area of pravastatin to that of the internal standard.

\[
\text{Amount (mg) of C}_2\text{H}_3\text{NaO}_7 = M_S \times Q_1 / Q_3 \times 4 \times 1.052
\]

\( M_S \): Amount (mg) of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

**Internal standard solution**—A solution of ethyl parahydroxybenzoate in the mixture of water and methanol (11:9) (3 in 4000).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, methanol, acetic acid (100) and triethylamine (550:450:1:1).

Flow rate: Adjust the flow rate so that the retention time of pravastatin is about 21 minutes.

**System suitability**—

System performance: When the procedure is run with 20 \( \mu \)L of the standard solution under the above operating conditions, the internal standard and pravastatin are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pravastatin to that of the internal standard is not more than 1.0%.

**Containers and storage**—Containers—Tight containers.

**Pravastatin Sodium Fine Granules**

プラバスタチンナトリウム細粒

Pravastatin Sodium Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of pravastatin sodium (\( C_{23}H_{35}NaO_7 \): 446.51).

**Method of preparation** Prepare fine particles as directed under Granules, with Pravastatin Sodium.

**Identification** To an amount of Pravastatin Sodium Fine Granules, equivalent to 10 mg of Pravastatin Sodium according to the labeled amount, add 20 mL of water, agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, and add water to 1 mL of the subsequent filtrate to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\): it exhibits a maximum between 237 nm and 241 nm.

**Purity** Related substances—The sample solution and the standard solution are stored at not exceeding 5°C after preparation. To an amount of Pravastatin Sodium Fine Granules, equivalent to 25 mg of Pravastatin Sodium according to the labeled amount, add 25 mL of a mixture of water and methanol (1:1), agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and methanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography \(<2.01>\) according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peaks, having the relative retention time of about 0.36 and about 1.9 to pravastatin, obtained from the sample solution is not larger than 1/2 and 3 times the peak area of pravastatin from the standard solution, respectively, the area of the peak other than pravastatin and the peaks mentioned above obtained from the sample solution is not larger than 1/5 times the peak area of pravastatin from the standard solution, and the total area of the peaks other than pravastatin obtained from the sample solution is not larger than 4.5 times the peak area of pravastatin from the standard solution. For this calculation, use the area of peaks, obtained by automatic integration method of related substances having the relative retention time of about 0.36, about 0.28 and about 0.88 to pravastatin, after multiplying by their relative response factors, 0.58, 0.86 and 0.82, respectively.

**Operating conditions**—

Detector: An ultraviolet spectrophotometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of water, methanol, acetic acid (100) and triethylamine (550:450:1:1).

Flow rate: 1.3 mL per minute.

Time span of measurement: For 75 minutes after injection, beginning after the solvent peak.

**System suitability**—

Test for required detectability: To exactly 1 mL of the standard solution add a mixture of water and methanol (1:1) to make exactly 10 mL. Confirm that the peak area of pravastatin obtained with 20 \( \mu \)L of this solution is equivalent to 7 to 13% of that with 20 \( \mu \)L of the standard solution.

System performance: When the procedure is run with 20 \( \mu \)L of the standard solution under the above operating con-
ditions, the number of theoretical plates and the symmetry factor of the peak of pravastatin are not less than 3500 and not more than 1.6, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pravastatin is not more than 1.5%.

**Uniformity of dosage units <6.02>** Perform the test according to the following method: the Pravastatin Sodium Fine Granules in single-unit container meets the requirement of the Content uniformity test.

To the total amount of the content of 1 container of Pravastatin Sodium Fine Granules add exactly V mL of the internal standard solution so that each mL contains 0.25 mg of pravastatin sodium (C_{23}H_{35}NaO_{7}), agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, pipet 2 mL of the subsequent filtrate add a mixture of water and methanol (1:1) to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of pravastatin sodium (C_{23}H_{35}NaO_{7})

\[ M_s = Q_s/\bar{Q} \times V/100 \times 1.052 \]

\( M_s \): Amount (mg) of pravastatin in taken Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

**Internal standard solution**—A solution of propyl parahydroxybenzoate in a mixture of water and methanol (1:1) (3 in 10,000).

**Dissolution <6.10>** When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Pravastatin Sodium Fine Granules is not less than 80%.

Start the test with an accurately weighed amount of Pravastatin Sodium Fine Granules, equivalent to about 5 mg of pravastatin sodium (C_{23}H_{35}NaO_{7}) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 23 mg of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS (separately determine the water <2.48> in the same manner as Pravastatin Sodium), and dissolve in a mixture of water and ethanol (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add a mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add a mixture of water and methanol (1:1) to make exactly 50 mL. Use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, \( Q_1 \) and \( Q_8 \), of the peak area of pravastatin to that of the internal standard.

Amount (mg) of pravastatin sodium (C_{23}H_{35}NaO_{7})

\[ M_s = Q_s/\bar{Q} \times 1/5 \times 1.052 \]

\( M_s \): Amount (mg) of pravastatin in taken Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

**Internal standard solution**—A solution of propyl parahydroxybenzoate in a mixture of water and methanol (1:1) (3 in 10,000).

**Operating conditions**—

Proceed as directed in the operating conditions in the Assay under Pravastatin Sodium.

**System suitability**—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and pravastatin are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pravastatin to that of the internal standard is not more than 1.0%.

**Containers and storage**—Container—Well-closed containers.

**Pravastatin Sodium Solution**

プラバスタチンナトリウム液

Pravastatin Sodium Solution contains not less than 95.0% and not more than 105.0% of the labeled amount of pravastatin sodium (C_{23}H_{35}NaO_{7}; 446.51).

**Method of preparation**—Prepare as directed under Liquids
and Solutions for Oral Administration, with Pravastatin Sodium.

**Identification** Pass a volume of Pravastatin Sodium Solution, equivalent to 1 mg of Pravastatin Sodium according to the labeled amount, through a column [5.5 mm in inside diameter, packed with 30 mg of divinylbenzene-N-vinyl pyrrolidone copolymer for column chromatography (30 μm in particle size), and washed with 1 mL of methanol and 1 mL of water]. Then wash with 1 mL of water, and elute with 1 mL of methanol. To 0.1 mL of the eluate add water to make 10 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry (2.2.4); it exhibits a maximum between 237 nm and 241 nm.

**pH** Being specified separately.

**Purity** Related substances—The sample solution and the standard solution are stored at not exceeding 15°C after preparation. To a volume of Pravastatin Sodium Solution, equivalent to 2 mg of Pravastatin Sodium according to the labeled amount, add a mixture of methanol and water (5:3) to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and methanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.2.1) according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peaks, having the relative retention time about 0.24 and about 0.85 to pravastatin, obtained from the sample solution is not larger than 3.5 times the peak area of pravastatin from the standard solution, the area of the peak other than pravastatin and the peaks mentioned above obtained from the sample solution is not larger than 2 times the peak area of pravastatin from the standard solution, the area of the peaks other than pravastatin and the peaks mentioned above obtained from the sample solution is not larger than 3/10 times the peak area of pravastatin from the standard solution.

**Operating conditions**—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of pravastatin, beginning after the solvent peak.

**System suitability**—

Test for required detectability: Pipet 2 mL of the standard solution, and add a mixture of water and methanol (1:1) to make exactly 10 mL. Confirm that the peak area of pravastatin obtained with 10 μL of this solution is equivalent to 15 to 25% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pravastatin are not less than 3000 and not more than 1.6, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pravastatin is not more than 2.5%.

**Uniformity of dosage units** (6.2) The solution in single-unit container meets the requirement of the Mass variation test.

**Microbial limit** (4.0) The acceptance criteria of TAMP and TYMC are 10² CFU/mL and 10³ CFU/mL, respectively. *Escherichia coli* is not observed.

**Assay** To a volume of Pravastatin Sodium Solution, equivalent to 2 mg of pravastatin sodium (C₂₃H₃₅NaO₇), add exactly 5 mL of the internal standard solution, add water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS (separately determine the water in the same manner as Pravastatin Sodium), and dissolve in a solution of disodium hydrogen phosphate dodecahydrate (1 in 200) to make exactly 50 mL. Pipet 6 mL of this solution, add exactly 5 mL of the internal standard solution, add water to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography (2.2.1) according to the following conditions. Calculate the ratios, Q₆ and Q₅, of the peak area of pravastatin to that of the internal standard.

\[
\text{Amount (mg) of pravastatin sodium} = M₃ \times \frac{Q₆}{Q₅} \times \frac{3}{25} \times 1.052
\]

\[M₃: \text{Amount (mg) of pravastatin in Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis}\]

**Internal standard solution**—A solution of ethyl parahydroxybenzoate in methanol (3 in 10,000).

**Operating conditions**—

Detector: An ultraviolet spectrophotometer (wavelength: 238 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, methanol, acetic acid (100) and triethylamine (500:500:1:1).

Flow rate: Adjust the flow rate so that the retention time of pravastatin is about 20 minutes.

**System suitability**—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and pravastatin are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pravastatin to that of the internal standard is not more than 1.0%.

**Containers and storage** Container—Tight containers.
**Pravastatin Sodium Tablets**

プラバスタチンナトリウム錠

Pravastatin Sodium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of pravastatin sodium (C_{23}H_{36}O_{7}NaO_{2}: 446.51).

**Method of preparation** Prepare as directed under Tablets, with Pravastatin Sodium.

**Identification** To a quantity of powdered Pravastatin Sodium Tablets, equivalent to 10 mg of Pravastatin Sodium according to the labeled amount, add 20 mL of water, agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, and add water to 1 mL of the subsequent filtrate to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 237 nm and 241 nm.

**Purity** Related substances—The sample solution and the standard solution are stored at not exceeding 15°C after preparation. To a quantity of powdered Pravastatin Sodium Tablets, equivalent to 50 mg of Pravastatin Sodium according to the labeled amount, add 40 mL of a mixture of water and methanol (1:1), agitate with the aid of ultrasonic waves, and centrifuge, then add a mixture of water and methanol (1:1) to make 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and methanol (1:1), agitate with the aid of ultrasonic waves, and centrifuge, then add a mixture of water and methanol (1:1) to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 237 nm and 241 nm.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

<table>
<thead>
<tr>
<th>Time after injection of sample (min)</th>
<th>Mobile phase A (vol%)</th>
<th>Mobile phase B (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>50 – 75</td>
<td>50 → 0</td>
<td>50 → 100</td>
</tr>
</tbody>
</table>

Flow rate: 1.3 mL per minute.

**System suitability**—
Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of water and methanol (1:1) to make exactly 10 mL. Confirm that the peak area of pravastatin obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pravastatin are not less than 3500 and not more than 1.6, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pravastatin is not more than 1.5%.

**Uniformity of dosage units <6.02>** Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Pravastatin Sodium Tablets, contain exactly 0.05 mL of the internal standard solution so that each mL contains 0.25 mg of pravastatin sodium (C_{23}H_{36}O_{7}NaO_{2}), agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. To 2 mL of the supernatant liquid add a mixture of water and methanol (1:1) to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of pravastatin sodium (C_{23}H_{36}O_{7})  
= \( M_z \times \frac{Q_z}{Q_s} \times \frac{V}{100} \times 1.052 \)

\( M_z \): Amount (mg) of pravastatin in Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

**Internal standard solution**—A solution of propyl parahydroxybenzoate in a mixture of water and methanol (1:1) (3 in 10,000).

**Dissolution <6.10>** When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Pravastatin Sodium Tablets is not less than 85%.

Start the test with 1 tablet of Pravastatin Sodium Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet \( V \) mL of the subsequent filtrate, add water to make exactly \( V \) mL so that each mL contains about 3.5 μg of pravastatin (C_{23}H_{36}O_{7}) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 23 mg of Pravastatin...
1,1,3,3-Tetramethylbutylammonium RS (separately determine the water in the same manner as Pravastatin Sodium), and dissolve in water to make exactly 100 mL. Pipet 3 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, $A_{11}$ and $A_{31}$, at 238 nm and $A_{12}$ and $A_{32}$ at 256 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of pravastatin sodium (C$_{23}$H$_{35}$NaO$_{7}$) is not more than 1.0%.

**Containers and storage** Container—Well-closed containers.

**Prazepam**

![Prazepam structure](image)

C$_{19}$H$_{17}$ClN$_{2}$O: 324.80

7-Chloro-1-(cyclopropylmethyl)-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one [2955-38-6]

Prazepam, when dried, contains not less than 98.5% of C$_{19}$H$_{17}$ClN$_{2}$O.

**Description** Prazepam occurs as white to light yellow crystals or crystalline powder. It is odorless. It is freely soluble in acetone, soluble in acetic anhydride, sparingly soluble in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

**Identification** (1) Dissolve 0.01 g of Prazepam in 3 mL of sulfuric acid, and observe under ultraviolet light (main wavelength: 365 nm): the solution shows a grayish blue fluorescence.

(2) Dissolve 0.01 g of Prazepam in 1000 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000). Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.26>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Prazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.01>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the Flame Coloration Tests <1.04> (2) with Prazepam: a green color appears.

**Melting point** <2.60> 145 – 148°C

**Purity** (1) Chloride <1.05>—To 1.0 g of Prazepam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. To 20 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Sulfate <1.14>—To 20 mL of the filtrate obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Prazep-
Prazepam Tablets

プラゼパム錠

Prazepam Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of prazepam (C19H17ClN2O: 324.80).

Method of preparation Prepare as directed under Tablets, with Prazepam.

Identification (1) To a quantity of powdered Prazepam Tablets, equivalent to 0.05 g of Prazepam according to the labeled amount, add 25 mL of acetone, shake well, and filter. Take 5 mL of the filtrate, evaporate on a water bath to dryness, and dissolve the residue in 3 mL of sulfuric acid. With this solution, proceed as directed in the Identification (1) under Prazepam.

(2) To a quantity of powdered Prazepam Tablets, equivalent to 0.02 g of Prazepam according to the labeled amount, add 200 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000), shake well, and filter. To 5 mL of the filtrate add a solution of sulfuric acid in ethanol (99.5) (3 in 1000) to make 50 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.40>. It exhibits maxima between 241 nm and 245 nm, between 283 nm and 287 nm and between 363 nm and 367 nm, and minima between 263 nm and 267 nm and between 334 nm and 338 nm.

Dissolution When the test is performed at 100 revolutions per minute according to the Basket method, using 900 mL of 0.1 mol/L hydrochloric acid TS as the dissolution medium, the dissolution rate in 30 minutes of Prazepam Tablets is not less than 80%.

Start the test with 1 tablet of Prazepam Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, measure exactly the subsequent V mL of the filtrate, add the dissolution medium to make exactly V mL so that each mL contains about 5 μg of prazepam (C19H17ClN2O) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 5 mg of prazepam for assay, previously dried at 105°C, for 2 hours, add 200 mL of the dissolution medium and dissolve with shaking, or by ultrasonication if necessary, add the dissolution medium to make exactly 1000 mL, and use this solution as the standard solution. Determine the absorbances, A1 and A9, of the sample solution and the standard solution at 240 nm as directed under Ultraviolet-visible Spectrophotometry <2.40>.

Dissolution rate (%) with respect to the labeled amount of prazepam (C19H17ClN2O) = M × A1/A9 × V/V × 1/C × 90
M: Amount (mg) of prazepam for assay
C: Labeled amount (mg) of prazepam (C19H17ClN2O) in 1 tablet

Assay Weigh accurately not less than 20 Prazepam Tablets, and powder. Weigh accurately a quantity of the powder, equivalent to about 50 mg of prazepam (C19H17ClN2O), add 30 mL of acetone, shake well, centrifuge, and separate the supernatant liquid. Repeat the same procedure twice with 30 mL each of acetone, combine all the supernatants liquid, and evaporate on a water bath to dryness. Dissolve the residue in 50 mL of a mixture of acetic anhydride and acetic acid (100:7:3), and titrate <2.50> with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS = 6.496 mg of C19H17ClN2O

Containers and storage Containers—Tight containers.
Prazosin Hydrochloride

プラゾシン塩酸塩

C_{19}H_{21}N_{5}O_{4}.HCl: 419.86
1-(4-Amino-6,7-dimethoxy-quinazolin-2-yl)-4-(2-furoyl)piperazine monohydrochloride [19237-84-4]

Prazosin Hydrochloride, when dried, contains not less than 97.0% and not more than 103.0% of C_{19}H_{21}N_{5}O_{4}.HCl.

Description Prazosin Hydrochloride occurs as a white crystalline powder.

It is slightly soluble in methanol, very slightly soluble in ethanol (99.5) and practically insoluble in water.

It gradually turns pale yellowish white on exposure to light.

Melting point: about 270°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Prazosin Hydrochloride in 0.01 mol/L hydrochloric acid-methanol TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Prazosin Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Prazosin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Prazosin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.1 g of Prazosin Hydrochloride add 5 mL of water and 1 mL of ammonia TS, shake, allow to stand for 5 minutes, and filter. Render the filtrate acid with acetic acid (100): the solution responds to the Qualitative Tests <1.09> for chlorides.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Prazosin Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 20 mg of Prazosin Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, and add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of prazosin from the sample solution is not larger than 2 times the peak area of prazosin from the standard solution, and the total area of the peaks other than the peak of prazosin from the sample solution is not larger than 5 times the peak area of prazosin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3.484 g of sodium 1-pentane sulfonate and 18 mL of tetramethylammonium hydroxide in 900 mL of water, adjust the pH to 5.0 with acetic acid (100), and add water to make 1000 mL. To this solution add 1000 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of prazosin is about 9 minutes.

Time span of measurement: About 6 times as long as the retention time of prazosin.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of prazosin obtained from 20 µL of this solution is equivalent to 35 to 65% of that of prazosin from 20 µL of the standard solution.

System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of prazosin are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of prazosin is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Loss on drying <2.4f> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.4g> Not more than 0.2% (1 g).

Assay Weigh accurately about 25 mg each of Prazosin Hydrochloride and Prazosin Hydrochloride RS, previously dried, and dissolve each in methanol to make exactly 50 mL. Pipet 3 mL each of these solutions, and add a mixture of methanol and water (7:3) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_T$ and $A_S$, of prazosin in each solution.

Amount (mg) of prazosin hydrochloride (C_{19}H_{21}N_{5}O_{4}.HCl) $M_S = M_S \times A_T/A_S$

$M_S$: Amount (mg) of Prazosin Hydrochloride RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter.
ter and 25 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol, water, acetic acid (100) and diethylamine (3500:1500:50:1).

Flow rate: Adjust the flow rate so that the retention time of prazosin is about 8 minutes.

**System suitability**

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of prazosin are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of prazosin is not more than 1.0%.

**Containers and storage**  Containers—Well-closed containers.

Storage—Light-resistant.

### Prednisolone

**プレドニゾロン**

\[
\text{C}_{21}\text{H}_{28}\text{O}_5: 360.44 \\
11\beta,17,21-\text{Trihydroxypregna-1,4-diene-3,20-dione} [50-24-8]
\]

Prednisolone, when dried, contains not less than 97.0% and not more than 102.0% of \(\text{C}_{21}\text{H}_{28}\text{O}_5\).

**Description** Prednisolone occurs as a white, crystalline powder.

It is soluble in methanol and in ethanol (95), slightly soluble in ethyl acetate and in chloroform, and very slightly soluble in water.

Melting point: about 235°C (with decomposition).

**Identification** (1) To 2 mg of Prednisolone add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

(2) Determine the infrared absorption spectrum of Prednisolone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry. Compare the spectrum with the Reference Spectrum or the spectrum of Prednisolone acetate, respectively, then evaporate the ethyl acetate to dryness, and repeat the test on the residues.

**Optical rotation** <2.49> \([\alpha]_D^{20}\text{ } +113 - +119^\circ\) (after drying, 0.2 g, ethanol (95), 20 mL, 100 mm).

**Purity** (1) Selenium—To 0.10 g of Prednisolone add 0.5 mL of a mixture of perchloric acid and sulfuric acid (1:1) and 2 mL of nitric acid, and heat on a water bath until no more brown gas evolves and the solution becomes to be a light yellow clear solution. After cooling, add 4 mL of nitric acid to this solution, then add water to make exactly 50 mL, and use this solution as the sample solution. Separately, pipet 3 mL of Standard Selenium Solution, add 0.5 mL of a mixture of perchloric acid and sulfuric acid (1:1) and 6 mL of nitric acid, then add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry according to the following conditions, and determine constant absorbances, \(A_T\) and \(A_S\), obtained on a recorder after rapid increasing of the absorption: \(A_T\) is smaller than \(A_S\) (not more than 30 ppm).

- Perform the test by using a hydride generating system and a thermal absorption cell.
- Lamp: A selenium hollow cathode lamp.
- Wavelength: 196.0 nm.
- Temperature of sample atomizer: When an electric furnace is used, about 1000°C.
- Carrier gas: Nitrogen or argon.

(2) Related substances—Dissolve 20 mg of Prednisolone in exactly 2 mL of a mixture of methanol and chloroform (1:1), and use this solution as the sample solution. Separately, dissolve 20 mg of hydrocortisone and 10 mg of prednisolone acetate each in a mixture of methanol and chloroform (1:1) to make exactly 100 mL, and use these solutions as the standard solution (1) and standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene and diethylamine (55:45:2) to a distance of about 15 cm, and air-dry the plate (do not dip the filter paper in the developing vessel). Spray evenly alkaline blue tetrazolium TS on the plate: the spots from the sample solution corresponding to those from the standard solutions (1) and (2), and no spots other than the principal spot, hydrocortisone and prednisolone acetate appear from the sample solution.

**Loss on drying** <2.41> Not more than 1.0% (0.5 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (0.5 g).

**Assay** Dissolve about 25 mg each of Prednisolone and Prednisolone RS, previously dried, and accurately weighed, in 50 mL of methanol, add exactly 25 mL of the internal standard solution to each, and add methanol to make 100 mL. To 1 mL each of these solutions add the mobile phase to make 10 mL, and use these solutions as the sample solution and standard solution. Perform the test with 20 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, \(Q_T\) and \(Q_S\), of the peak area of prednisolone to that of the internal standard.

Amount (mg) of \(\text{C}_{21}\text{H}_{28}\text{O}_5 = M_S \times Q_T/Q_S\)
Prednisolone Tablets / Official Monographs

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**Prednisolone Tablets**

Prednisolone Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of prednisolone (C21H28O5: 360.44).  

**Method of preparation**  
Prepare as directed under Tablets, with Prednisolone.

**Identification (1)**  
Weigh a quantity of powdered Prednisolone Tablets equivalent to 0.05 g of Prednisolone according to the labeled amount, add 10 mL of chloroform, shake for 15 minutes, and filter. Evaporate the filtrate on a water bath to dryness. Dry the residue at 105°C for 1 hour, and proceed as directed in the Identification (1) under Prednisolone.

(2) Determine the infrared absorption spectra of the residue obtained in (1) and Prednisolone RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2,2.25>: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears, dissolve the sample and the RS in ethyl acetate, evaporate to dryness, and repeat the test on the residues.

**Uniformity of dosage units**  
Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Transfer 1 tablet of Prednisolone Tablets to a volumetric flask, and shake with 10 mL of water until the tablet is disintegrated. Add 50 mL of methanol, shake for 30 minutes, and add methanol to make exactly 100 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, and add methanol to make exactly V mL to provide a solution that contains about 10 μg of prednisolone (C21H28O5) per mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Prednisolone RS, previously dried at 105°C for 3 hours, dissolve in 10 mL of water and 50 mL of methanol, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_I and A_S, of the sample solution and standard solution at 242 nm as directed under Ultraviolet-visible Spectrophotometry <2,2.25>.

\[
\text{Amount (mg) of prednisolone (C21H28O5)} = M_S \times \frac{A_I}{A_S} \times \frac{V}{V} \times \frac{1}{10}
\]

**Dissolution**  
When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 20 minutes of Prednisolone Tablets is not less than 70%.

Start the test with 1 tablet of Prednisolone Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 10 mg of Prednisolone RS, previously dried at 105°C for 3 hours, and dissolve in ethanol (95) to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_I and A_S, of the sample solution and standard solution at the maximum wavelength at about 242 nm as directed under Ultraviolet-visible Spectrophotometry <2,2.25>, using water as the blank.

\[
\text{Dissolution rate (%)} = \frac{M_S \times \frac{A_I}{A_S} \times \frac{V}{V} \times \frac{1}{10}}{C}
\]

**Assay**  
Weigh accurately and powder not less than 20 Prednisolone Tablets using an agate mortar. Weigh accurately a portion of the powder, equivalent to about 5 mg of prednisolone (C21H28O5), add 1 mL of water, and shake gently. Add exactly 5 mL of the internal standard solution and 15 mL of methanol, and shake vigorously for 20 minutes. To 1 mL of this solution add the mobile phase to make 10 mL, and filter through a membrane filter with pore size of 0.45 μm. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Prednisolone RS, previously dried at 105°C for 3 hours, dissolve in 50 mL of methanol, add exactly 25 mL of the internal standard solution, and add methanol to make 100 mL. To 1 mL of this solution add the mobile phase to make 10 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Prednisolone with these solutions.
Prednisolone Acetate

### Description

Prednisolone Acetate occurs as a white, crystalline powder. It is slightly soluble in methanol, in ethanol (95), in chloroform, and practically insoluble in water. Melting point: about 235°C (with decomposition).

#### Identification

1. **Optical rotation** $\langle 2.49 \rangle$  
   $\alpha^2_{D} = [128^\circ - 137^\circ]$ (after drying, 70 mg, methanol, 20 mL, 100 mm).

2. **Purity** Related substances—Dissolve 0.20 g of Prednisolone Acetate in exactly 10 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Separately, dissolve 20 mg each of prednisolone, cortisone acetate and hydrocortisone acetate in exactly 10 mL of a mixture of chloroform and methanol (9:1). Pipet 1 mL of this solution, add a mixture of chloroform and methanol (9:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 5 $\mu$L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, diethyl ether, methanol and water (385:75:40:6) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (wavelength: 254 nm): the spots from the sample solution corresponding to those from the standard solution are not more intense than the spots from the standard solution, and any spot from the sample solution other than the principal spot and the spots from prednisolone, cortisone acetate and hydrocortisone acetate does not appear.

#### Loss on drying $\langle 2.4I \rangle$

Not more than 1.0% (0.5 g, 105°C, 3 hours).

#### Residue on ignition $\langle 2.44 \rangle$

Not more than 0.1% (0.5 g).

#### Assay

Dissolve about 10 mg each of Prednisolone Acetate and Prednisolone Acetate RS, previously dried and accurately weighed, in 60 mL each of methanol, add exactly 2 mL each of the internal standard solution, then add methanol to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.0I \rangle$ according to the following conditions, and calculate the ratios, $Q_2$ and $Q_3$, of the peak height of prednisolone acetate to that of the internal standard.

$$Q_2 = \frac{M_2 \times Q_2}{S_2}, \quad Q_3 = \frac{M_3 \times Q_3}{S_3}$$

where $M_2$ and $M_3$ are the amounts of prednisolone acetate and prednisolone acetate RS, respectively, evaporate to dryness, and repeat the test on the residues.

#### System suitability—

**System performance:** When the procedure is run with 10 $\mu$L of the standard solution under the above operating conditions, prednisolone acetate and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

**System repeatability:** When the test is repeated 6 times with 10 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of prednisolone acetate to that of the internal standard is not more than 1.0%.

### Containers and storage

Containers—Tight containers.
Prednisolone Sodium Phosphate

prednisolone sodium phosphate: 484.39
11β,17,21-Trihydroxyprogna-1,4-diene 3,20-dione
21-(disodium phosphate)

Prednisolone Sodium Phosphate contains not less than 97.0% and not more than 103.0% of C$_{21}$H$_{27}$Na$_2$O$_{5}$P, calculated on the anhydrous basis.

**Description** Prednisolone Sodium Phosphate occurs as a white to pale yellow powder. It is freely soluble in water, soluble in methanol, and practically insoluble in ethanol (99.5). It is hygroscopic.

**Identification** (1) Moisten 1.0 g of Prednisolone Sodium Phosphate with a small amount of sulfuric acid, and gradually heat to incinerate. After cooling, dissolve the residue in 10 mL of dilute nitric acid, and heat in a water bath for 30 minutes. After cooling, filter if necessary. This solution responds to the Qualitative Tests <1.09> for phosphate.

(2) Dissolve 2 mg of Prednisolone Sodium Phosphate in 2 mL of sulfuric acid, and allow to stand for 2 minutes: a deep red color, without fluorescence, develops.

(3) Determine the absorption spectrum of a solution of Prednisolone Sodium Phosphate (1 in 50,000) as directed under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Prednisolone Sodium Phosphate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(5) The solution obtained in (1) responds to the Qualitative Tests <1.09> for sodium salt.

**Optical rotation** <2.49> $\sigma$$_D$$^{20}$ +96 to +103° (1 g, calculated on the anhydrous basis, phosphate buffer solution, pH 7.0, 100 mL, 10 mm).

**pH** <2.3> Dissolve 1.0 g of Prednisolone Sodium Phosphate in 100 mL of water: the pH of the solution is between 7.5 and 9.0.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Prednisolone Sodium Phosphate in 10 mL of water: the solution is clear and not more colored than the following control solution.

Control solution: To a mixture of 3.0 mL of Cobalt (II) Chloride CS, 3.0 mL of Iron (III) Chloride CS and 2.4 mL of Copper (II) Sulfate CS add diluted hydrochloric acid (1 in 40) to make 10 mL. To 2.5 mL of this solution add diluted hydrochloric acid (1 in 40) to make 100 mL.

(2) Heavy metals <1.07>—Proceed with 0.5 g of Prednisolone Sodium Phosphate according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 40 ppm).

(3) Free phosphoric acid—Weigh accurately about 0.25 g of Prednisolone Sodium Phosphate, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Pipet 5 mL of each of the sample solution and Phosphoric Acid Standard Solution, add 2.5 mL of hexammonium heptamolybdate-sulfuric acid TS and 1 mL of l-amino-2-naphthol-4-sulfonic acid TS, shake, add water to make exactly 25 mL, and allow to stand at 20 ± 1°C for 30 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 5 mL of water in the same manner as the blank. Determine the absorbances, $A_T$ and $A_5$, of each solution from the sample solution and standard solution at 740 nm: the content of free phosphoric acid is not more than 1.0%.

Content (% of free phosphoric acid (H$_3$PO$_4$) = 1/M × $A_T/A_5$ × 258.0

$M$: Amount (mg) of Prednisolone Sodium Phosphate, calculated on the anhydrous basis

(4) Related substances—Dissolve 10 mg of Prednisolone Sodium Phosphate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of prednisolone phosphate from the sample solution is not larger than 1.5 times the peak area of prednisolone phosphate from the standard solution, and the total area of the peaks other than the peak of prednisolone phosphate from the sample solution is not larger than 2.5 times the peak area of prednisolone phosphate from the standard solution.

**Operating conditions**—
Detector: An ultraviolet absorption photometer (wavelength: 245 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilated silica gel for liquid chromatography (3 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 2.5 with phosphoric acid. To 1000 mL of this solution add 250 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of prednisolone phosphate is about 7 minutes.
Time span of measurement: About 4 times as long as the retention time of prednisolone phosphate.
System suitability—
Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of prednisolone phosphate ob-
tained from 20 μL of this solution is equivalent to 7 to 13% of that of prednisolone phosphate from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of prednisolone phosphate are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of prednisolone phosphate is not more than 2.0%.

(5) Residual solvent—Being specified separately.

**Water** <2.49> Not more than 8.0% (0.1 g, volumetric titration, direct titration).

**Assay** Weigh accurately about 0.1 g of Prednisolone Sodium Phosphate, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add 1 mL of alkaline phosphatase TS, and allow to stand for 2 hours with occasional shaking. To this solution add exactly 20 mL of 1-octanol, and shake vigorously. Centrifuge this solution, pipet 10 mL of the 1-octanol layer, add 1-octanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Prednisolone RS, previously dried at 105°C for 3 hours, and dissolve in 1-octanol to make exactly 100 mL. Pipet 6 mL of this solution, add a solution prepared by adding 1 mL of alkaline phosphatase TS to 2 mL of water and being allowed to stand for 2 hours with occasional gentle shaking, add exactly 14 mL of 1-octanol, and shake vigorously. Proceed in the same manner as the sample solution to make the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using 1-octanol as the blank, and determine the absorbances, $A_T$ and $A_S$, at 245 nm.

Amount (mg) of prednisolone sodium phosphate
\[
(C_{21}H_{27}Na_2O_8P) = M_S 	imes A_T/A_S \times 3 \times 1.344
\]

$M_S$: Amount (mg) of Prednisolone RS

**Containers and storage** Containers—Tight containers.

### Prednisolone Succinate

プレドニゾロンコハク酸エステル

C₂₅H₃₂O₈: 460.52
11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione
21-(hydrogen succinate)
[2920-86-7]

Prednisolone Succinate, when dried, contains not less than 97.0% and not more than 103.0% of C₂₅H₃₂O₈.

**Description** Prednisolone Succinate occurs as a white, fine, crystalline powder. It is odorless. It is freely soluble in methanol, soluble in ethanol (95%), and very slightly soluble in water and in diethyl ether.

Melting point: about 205°C (with decomposition).

**Identification** (1) To 2 mg of Prednisolone Succinate add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

(2) Determine the infrared absorption spectrum of Prednisolone Succinate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Prednisolone Succinate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> $[α]_D^{2920}: +114° +120°$ (after drying, 67 mg, methanol, 10 mL, 100 mm).

**Purity** Related substances—Dissolve 0.10 g of Prednisolone Succinate in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 30 mg of prednisolone in methanol to make exactly 10 mL. Pipet 1 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and ethanol (95) (2:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.47> Not more than 0.5% (1 g, in vacuum, phosphorus(V) oxide, 60°C, 6 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 10 mg each of Prednisolone Succinate and Prednisolone Succinate RS, previously dried, and dissolve each in methanol to make exactly 100 mL. Pipet 5 mL each of these solutions, add methanol to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and standard solution at 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of C₂₅H₃₂O₈ = $M_S \times A_T/A_S$

$M_S$: Amount (mg) of Prednisolone Succinate RS

**Containers and storage** Containers—Tight containers.
Prednisolone Sodium Succinate for Injection

注射用ブレドニゾロンホルム酸エステルナトリウム

C₂₅H₃₁NaO₈: 482.50
Monosodium 11β,17,21-trihydroxypregna-1,4-diene-3,20-dione 21-succinate [1715-33-9]

Prednisolone Sodium Succinate for Injection is a preparation for injection which is dissolved before use.

It contains not less than 72.4% and not more than 83.2% of prednisolone sodium succinate (C₂₅H₃₁NaO₈) and the equivalent of not less than 90.0% and not more than 110.0% of the labeled amount of prednisolone (C₂₅H₃₃O₈: 360.44).

The amount should be stated as the amount of prednisolone (C₂₅H₃₃O₈).

Method of preparation Prepare as directed under Injection, with Prednisolone Succinate and Dried Sodium Carbonate or Sodium Hydroxide.

It contains a suitable buffer agent.

Description Prednisolone Sodium Succinate for Injection occurs as a white powder or porous, friable mass.

It is freely soluble in water.

It is hygroscopic.

Identification (1) To 2 mg of Prednisolone Sodium Succinate for Injection add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

(2) Dissolve 0.01 g of Prednisolone Sodium Succinate for Injection in 1 mL of methanol, add 1 mL of Fehling’s TS, and heat: an orange to red precipitate is formed.

(3) Dissolve 0.1 g of Prednisolone Sodium Succinate for Injection in 2 mL of sodium hydroxide TS, allow to stand for 10 minutes, and filter. Add 1 mL of dilute hydrochloric acid to the filtrate, shake, and filter if necessary. Adjust the solution with diluted ammonia TS (1 in 10) to a pH of about 6, and add 2 to 3 drops of iron (III) chloride TS: a brown precipitate is formed.

(4) Prednisolone Sodium Succinate for Injection responds to the Qualitative Tests <1.09> (1) for sodium salt.

pH <2.54> Dissolve 1.0 g of Prednisolone Sodium Succinate for Injection in 40 mL of water: the pH of the solution is between 6.5 and 7.2.

Purity Clarity and color of solution—Dissolve 0.25 g of Prednisolone Sodium Succinate for Injection in 10 mL of water: the solution is clear and colorless.

Loss on drying <2.4> Not more than 2.0% (0.15 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Bacterial endotoxins <4.0> Less than 2.4 EU/mg of prednisolone (C₂₅H₃₃O₈).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take a quantity of sealed containers of Prednisolone Sodium Succinate for Injection, equivalent to about 0.1 g of prednisolone (C₂₅H₃₃O₈), and dissolve the contents in a suitable amount of diluted methanol (1 in 2), and transfer to a 100-mL volumetric flask. Wash each container with diluted methanol (1 in 2), collect the washings in the volumetric flask, and add diluted methanol (1 in 2) to make volume. Pipet 4 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, mix, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Prednisolone Succinate RS, previously dried in a desiccator for 6 hours (in vacuum, phosphorus (V) oxide, 60°C), dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, mix, and use this solution as the standard solution. Perform the test with 10 μL of the sample solution and standard solution as directed under Liquid Chromatography according <2.07> to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of prednisolone succinate to that of the internal standard.

\[
\text{Amount (mg) of prednisolone sodium succinate (C₂₅H₃₃NaO₈)} = M_S \times Q_1 / Q_2 \times 5 \times 0.1048
\]

\[
\text{Amount (mg) of prednisolone (C₂₁H₂₇O₃)} = M_S \times Q_1 / Q_2 \times 5 \times 0.783
\]

\[
M_S: \text{Amount (mg) of Prednisolone Succinate RS}
\]

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted methanol (1 in 2) (1 in 25,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.32 g of tetra n-butylammonium bromide, 3.22 g of disodium hydrogen phosphate dodecahydrate and 6.94 g of potassium dihydrogen phosphate in 1000 mL of water. To 840 mL of this solution add 1160 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of prednisolone succinate is about 15 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, prednisolone succinate and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of prednisolone succinate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Primidone

プリミドン

C₁₂H₁₄N₂O₂: 218.25
5-Ethyl-5-phenyl-2,3-dihydropyrimidine-4,6(1H,5H)-dione [125-33-7]

Primidone, when dried, contains not less than 98.5% of C₁₂H₁₄N₂O₂.

Description Primidone occurs as a white, crystalline powder or granules. It is odorless and has a slightly bitter taste.
It is soluble in N,N-dimethylformamide, sparingly soluble in pyridine, slightly soluble in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

Identification (1) Heat 0.5 g of Primidone with 5 mL of diluted sulfuric acid (1 in 2): the odor of formaldehyde is perceptible.
(2) Mix 0.2 g of Primidone with 0.2 g of anhydrous sodium carbonate, and heat: the gas evolved changes moistened red litmus paper to blue.

Melting point <2.60> 279 – 284°C

Purity (1) Clarity of color of solution—Dissolve 0.10 g of Primidone in 10 mL of N,N-dimethylformamide: the solution is clear and colorless.
(2) Heavy metals <1.07>—Proceed with 2.0 g of Primidone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).
(3) 2-Ethyl-2-phenylmalonediamide—Dissolve 0.10 g of Primidone in 2 mL of pyridine, add exactly 2 mL of the internal standard solution, then add 1 mL of bis-trimethylsilyl acetamide, shake well, and heat at 100°C for 5 minutes. Cool, add pyridine to make 10 mL, and use this solution as the sample solution. Separately, dissolve 50 mg of 2-ethyl-2-phenylmalonediamide in pyridine to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, proceed in the same manner as Primidone, and use this solution as the standard solution. Perform the test with 2 μL of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q₁ and Q₂, of the peak area of 2-ethyl-2-phenylmalonediamide to that of the internal standard: Q₁ is not more than Q₂.

Internal standard solution—A solution of stearylalcohol in pyridine (1 in 2000).

Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A glass column 3 mm in inside diameter and 150 cm in length, packed with siliceous earth for gas chromatography (125 to 150 μm in particle diameter) coated with 50% phenyl-methyl silicon polymer for gas chromatography at the ratio of 3%.
Column temperature: A constant temperature of about 195°C.
Carrier gas: Nitrogen.
Flow rate: Adjust the flow rate so that the retention time of stearylalcohol is about 10 minutes.

System suitability—
System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, 2-ethyl-2-phenylmalonediamide and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.
System repeatability: When the test is repeated 5 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of 2-ethyl-2-phenylmalonediamide to that of the internal standard is not more than 1.5%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 20 mg each of Primidone and Primidone RS, previously dried, dissolve each in 20 mL of ethanol (95) by warming, and after cooling, add ethanol (95) to make exactly 25 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbance, A₁, of the sample solution and standard solution at the wavelength of maximum absorption at about 257 nm, and the absorbances, A₂ and A₃, at the wavelength of minimum absorption at about 254 nm and at about 261 nm, as directed under Ultraviolet-visible Spectrophotometry <2.24>, using ethanol (95) as the blank.

Amount (mg) of C₁₂H₁₄N₂O₂ = Mₛ × (2A₁ – A₂ – A₃)/[(2A₁ – A₂ – A₃)ₙ]

Mₛ: Amount (mg) of Primidone RS
where, (2A₁ – A₂ – A₃)ₙ is the value from the sample solution, and (2A₁ – A₂ – A₃)ₙ is from the standard solution.

Containers and storage Containers—Tight containers.
Probenecid

プロベネシド

\[
\text{C}_{13}\text{H}_{19}\text{NO}_4\text{S}: 285.36
\]

4-(Dipropylaminosulfonyl)benzoic acid

[57-66-9]

Probenecid, when dried, contains not less than 98.0% of \( \text{C}_{13}\text{H}_{19}\text{NO}_4\text{S} \).

**Description** Probenecid occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste, followed by unpleasant bitter.

It is sparingly soluble in ethanol (99.5), and practically insoluble in water.

It dissolves in sodium hydroxide TS and in ammonia TS.

Melting point: 198 – 200°C

**Identification (1)** Heat Probenecid strongly: the odor of sulfur dioxide is perceptible.

(2) Determine the absorption spectrum of a solution of Probenecid in ethanol (99.5) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Probenecid RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity (1)** Acidity—To 2.0 g of Probenecid add 100 mL of water, heat on a water bath with occasional shaking for 30 minutes, cool, and filter. To the filtrate add 1 drop of phenolphthalein TS and 0.50 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(2) Chloride \(<1.02>\)—To 1.0 g of Probenecid add 100 mL of water and 1 mL of nitric acid, and heat on a water bath with occasional shaking for 30 minutes. After cooling, add, if necessary, water to make 100 mL, and filter. Perform the test using 50 mL of the filtrate as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate \(<1.14>\)—To 1.0 g of Probenecid add 100 mL of water and 1 mL of hydrochloric acid, and heat on a water bath with occasional shaking for 30 minutes. After cooling, add, if necessary, water to make 100 mL, and filter. Perform the test using 50 mL of the filtrate as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuriac acid VS (not more than 0.038%).

(4) Heavy metals \(<1.07>\)—Proceed with 2.0 g of Probenecid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic \(<1.17>\)—Prepare the test solution with 1.0 g of Probenecid according to Method 3, and perform the test (not more than 2 ppm).

**Loss on drying** \(<2.41>\) Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** \(<2.44>\) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Probenecid, previously dried, and dissolve in 50 mL of neutralized ethanol. Titrate \(<2.50>\) with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS is equivalent to 28.54 mg of \( \text{C}_{13}\text{H}_{19}\text{NO}_4\text{S} \).

**Containers and storage** Containers—Well-closed containers.

**Probenecid Tablets**

Probenecid Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of probenecid (\( \text{C}_{13}\text{H}_{19}\text{NO}_4\text{S}: 285.36 \)).

**Method of preparation** Prepare as directed under Tablets, with Probenecid.

**Identification (1)** Weigh a quantity of powdered Probenecid Tablets, equivalent to 0.5 g of Probenecid according to the labeled amount, add 50 mL of ethanol (95) and 1 mL of 1 mol/L hydrochloric acid TS, shake, and filter. Evaporate the filtrate on a water bath to about 20 mL. After cooling, collect produced crystals, recrystallize with 50 mL of dilute ethanol, and dry at 105°C for 4 hours: it melts \(<2.60>\) between 196°C and 200°C. With the crystals so obtained, proceed as directed in the Identification (1) under Probenecid.

(2) Determine the absorption spectrum of a solution of the dried crystals obtained in (1) in ethanol (99.5) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Probenecid RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Uniformity of dosage units** \(<2.02>\) Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Probenecid Tablets add 30 mL of water and 2 mL of 1 mol/L hydrochloric acid TS, treat with ultrasonic waves with occasional shaking to disintegrate the tablet completely, and add ethanol (99.5) to make exactly 100 mL. Centrifuge this solution, pipet 3 mL of the supernatant liquid, and add 1 mL of 1 mol/L hydrochloric acid TS and ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of this solution, and add ethanol (99.5) to make exactly \( V\) mL so that each mL contains about 15 \(\mu\)g of probenecid (\( \text{C}_{13}\text{H}_{19}\text{NO}_4\text{S} \)), and use this solution as the sample solution. Separately, weigh accurately about 0.125 g of Probenecid RS, previously dried at 105°C for 4 hours, dissolve in 15 mL of water, 1 mL of 1 mol/L hydrochloric acid TS and ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of this solution, and add ethanol (99.5) to make exactly \( V\) mL so that each mL contains about 15 \(\mu\)g of probenecid (\( \text{C}_{13}\text{H}_{19}\text{NO}_4\text{S} \)), and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry \(<2.24>\), using a solution, prepared by adding...
ethanol (99.5) to 1 mL of 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, as the blank, and determine the absorbances, $A_T$ and $A_S$, at 248 nm.

$$\text{Amount (mg) of probenecid (C}_13\text{H}_19\text{NO}_4\text{S}) = M_S \times A_T/A_S \times 25$$

$M_S$: Amount (mg) of Probucol RS

**Dissolution** 6.2.10 When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of the 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Probucol Tablets is not less than 80%.

Start the test with 1 tablet of Probucol Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet $V$ mL of the subsequent filtrate, add the dissolution medium to make exactly $V$ mL so that each mL contains about 14 μg of probenecid (C$_{13}$H$_{19}$NO$_4$S) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 70 mg of Probucol RS, previously dried at 105°C, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, at 244 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry 2.24v.

Dissolution rate (%) with respect to the labeled amount of probenecid (C$_{13}$H$_{19}$NO$_4$S)

$$= M_S \times A_T/A_S \times V/V \times 1/C \times 18$$

$M_S$: Amount (mg) of Probucol RS

**Assay** Weigh accurately, and powder not less than 20 Probucol Tablets. Weigh accurately a portion of the powder, equivalent to about 0.25 g of probenecid (C$_{13}$H$_{19}$NO$_4$S), add 30 mL of water and 2 mL of 1 mol/L hydrochloric acid TS, shake, add 30 mL of ethanol (99.5), disperse the particles with the aid of ultrasonic waves, and add ethanol (99.5) to make exactly 100 mL. Centrifuge the solution, pipet 3 mL of the supernatant liquid, add 1 mL of 1 mol/L hydrochloric acid TS, and add ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of the solution, add ethanol (99.5) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.125 g of Probucol RS, previously dried at 105°C for 4 hours, add 15 mL of water and 1 mL of 1 mol/L hydrochloric acid TS, then add ethanol (99.5) to make exactly 50 mL. Pipet 3 mL of this solution, add 1 mL of 1 mol/L hydrochloric acid TS, and add ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of the solution, add ethanol (99.5) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and standard solution at 248 nm as directed under Ultraviolet-visible Spectrophotometry 2.24v, using a solution, prepared by mixing 1 mL of 0.1 mol/L hydrochloric acid TS and sufficient ethanol (99.5) to make exactly 50 mL, as the blank.

$$\text{Amount (mg) of probenecid (C}_13\text{H}_19\text{NO}_4\text{S}) = M_S \times A_T/A_S \times 2$$

$M_S$: Amount (mg) of Probucol RS

**Containers and storage** Containers—Well-closed containers.

**Probucol**

![Probucol structure](image)

C$_{31}$H$_{48}$O$_2$S$_2$: 516.84
4,4’-[Propan-2,2-diylbis(sulfandiyl)]bis[2,6-bis(1,1-dimethylphenyl)phenol]
[23289-49-5]

Probucol, when dried, contains not less than 98.5% and not more than 101.0% of C$_{31}$H$_{48}$O$_2$S$_2$.

**Description** Probucol occurs as a white crystalline powder.

It is very soluble in tetrahydrofuran, freely soluble in ethanol (99.5), soluble in methanol, and practically insoluble in water.

It gradually turns light yellow on exposure to light.

**Identification** (1) Determine the absorption spectrum of a solution of Probucol in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry 2.24v, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Probucol RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Probucol as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25v, and compare the spectrum with the Reference Spectrum or the spectrum of Probucol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** 2.6.10 125 – 128°C

**Purity** (1) Heavy metals 1.079—Proceed with 2.0 g of Probucol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 0.40 g of Probucol in 5 mL of ethanol (99.5), add the mobile phase to make 20 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, and add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography 2.01 according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak having the relative retention time of about 0.9 with respect to probucol from the sample solution is not larger than the peak area of probucol from the standard solution; the area of peak hav-
ing the relative retention time of about 1.9 with respect to probucol from the sample solution is not larger than 25 times the peak area of probucol from the standard solution; and the area of each peak other than the peak of probucol and other than the peaks mentioned above is not larger than 5 times the peak area of probucol from the standard solution. Furthermore, the total area of the peaks other than probucol from the sample solution is not larger than 50 times the peak area of probucol from the standard solution. For this calculation, use the areas of the peaks, having the relative retention times of about 0.9 and about 1.9 with respect to probucol, after multiplying by their relative response factors, 1.2 and 1.4, respectively.

**Operating conditions—**

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of probucol, beginning after the solvent peak, excluding the peak having the relative retention time of about 0.5 with respect to probucol.

**System suitability—**

Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of probucol obtained from 5 μL of this solution is equivalent to 14 to 26% of that of probucol from 5 μL of the standard solution.

System performance: To 1 mL of the sample solution add the mobile phase to make 50 mL. To 1 mL of this solution add 1 mL of a solution of phthalic acid bis(cis-3,3,5-trimethylcyclohexyl) in the mobile phase (1 in 1000), 5 mL of acetonitrile and water (93:7), and the mobile phase to make 20 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, phthalic acid bis(cis-3,3,5-trimethylcyclohexyl) and probucol are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of probucol is not more than 5%.

(3) Residual solvent—Being specified separately.

**Loss on drying** <2.41> Not more than 0.5% (1 g, in vacuum, 80°C, 1 hour).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 60 mg each of Probucol and Probucol RS, previously dried, dissolve each in 5 mL of tetrahydrofuran, and add the mobile phase to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution and the mobile phase to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q₃ and Q₅, of the peak area of probucol to that of the internal standard.

\[
M₃ = Mₛ \times Qₛ/ Q₃
\]

**Internal standard solution**—Dissolve 0.2 g of bis(cis-3,3,5-trimethylcyclohexyl) phthalate in 1 mL of tetrahydrofuran, and add the mobile phase to make 50 mL.

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wave-length: 242 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of acetonitrile and water (93:7).

Flow rate: Adjust the flow rate so that the retention time of probucol is about 13 minutes.

**System suitability—**

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and probucol are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of peak area of probucol to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

**Probucol Fine Granules**

**Procalcitol 細粒**

Probucol Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of probucol (C₃₁H₄₈O₂S₂: 516.84).

**Method of preparation** Prepare as directed under Granules, with Probucol.

**Identification** To an amount of pulverized Probucol Fine Granules, equivalent to 50 mg of Probucol according to the labeled amount, add 100 mL of methanol, shake, and filter. To 2 mL of the filtrate add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>:

- It exhibits a maximum between 240 nm and 244 nm.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: the granules in single-unit containers meet the requirement of the Content uniformity test.

To the total amount of the content of 1 container of Probucol Fine Granules add 70 mL of methanol, shake thoroughly, and add methanol to make exactly 100 mL. Centrifuge, pipet 1 V mL of the supernatant liquid, equivalent to about 5 mg of probucol (C₃₁H₄₈O₂S₂), add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

\[
M₅ = Mₛ \times Qₛ/ Q₅ \times 10/V
\]

**Internal standard solution**—A solution of bis(cis-3,3,5-trimethylcyclohexyl) phthalate in methanol (1 in 250).
Particle size &lt;6.02&gt; It meets the requirements of Fine granules.

Assay Weigh accurately an amount of pulverized Probucol Fine Granules, equivalent to about 0.25 g of probucol \((C_{31}H_{48}O_{2}S_{2})\), add 70 mL of methanol, shake thoroughly, and add methanol to make exactly 100 mL. Centrifuge, pipet 2 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Probucol RS, previously dried under reduced pressure at 80°C for 1 hour, and dissolve in methanol to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 10 µL of each of the sample solution and standard solution as directed under Liquid Chromatography &lt;2.01&gt; according to the following conditions, and calculate the ratios, \(Q_t\) and \(Q_s\), of the peak area of probucol to that of the internal standard.

\[
\text{Amount (mg) of probucol} = M_6 \times Q_t / Q_s \times 5
\]

\(M_5\): Amount (mg) of Probucol RS

Internal standard solution—A solution of bis\((\text{cis}-3,3,5\text{-trimethylcyclohexyl})\) phthalate in methanol (1 in 250).

Operating conditions—
Detector, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Probucol.
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

System suitability—
System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the internal standard and probucol are eluted in this order with the resolution between these peaks being not less than 3.
System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of probucol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Probucol Tablets

プロブコール錠

Probucol Tablets contain not less than 95.0% and not more than 105.0% of probucol \((C_{31}H_{48}O_{2}S_{2}): 516.84\).

Method of preparation Prepare as directed under Tablets, with Probucol.

Identification To an amount of pulverized Probucol Tablets, equivalent to 50 mg of Probucol according to the labeled amount, add 100 mL of methanol, shake, and filter. To 2 mL of the filtrate add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry &lt;2.242&gt;: it exhibits a maximum between 240 nm and 244 nm.

Uniformity of dosage units &lt;6.02&gt; Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Shake 1 tablet of Probucol Tablets with a suitable amount of methanol until the tablet is disintegrated, and add methanol to make exactly \(V\) mL so that each mL of the solution contains about 2.5 mg of probucol \((C_{31}H_{48}O_{2}S_{2})\). Centrifuge the solution, pipet 2 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, then add methanol to make 100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

\[
\text{Amount (mg) of probucol} = M_6 \times Q_t / Q_s \times V/20
\]

\(M_5\): Amount (mg) of Probucol RS

Internal standard solution—A solution of bis\((\text{cis}-3,3,5\text{-trimethylcyclohexyl})\) phthalate in methanol (1 in 250).

Disintegration &lt;6.09&gt; It meets the requirement.

Assay Weigh accurately the mass of 20 Probucol Tablets, and powder the tablets. Weigh accurately a portion of the powder, equivalent to about 0.25 g of probucol \((C_{31}H_{48}O_{2}S_{2})\), add 70 mL of methanol, shake thoroughly, and add methanol to make exactly 100 mL. Centrifuge, pipet 2 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Probucol RS, previously dried under reduced pressure at 80°C for 1 hour, and dissolve in methanol to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 10 µL of each of the sample solution and standard solution as directed under Liquid Chromatography &lt;2.01&gt; according to the following conditions, and calculate the ratios, \(Q_t\) and \(Q_s\), of the peak area of probucol to that of the internal standard.

\[
\text{Amount (mg) of probucol} = M_6 \times Q_t / Q_s \times 5
\]

\(M_5\): Amount (mg) of Probucol RS

Internal standard solution—A solution of bis\((\text{cis}-3,3,5\text{-trimethylcyclohexyl})\) phthalate in methanol (1 in 250).

System suitability—
Detector, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Probucol.
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

System performance: When the procedure is run with 10 µL of the sample solution and standard solution as directed under Liquid Chromatography &lt;2.01&gt; according to the following conditions, and calculate the ratios, \(Q_t\) and \(Q_s\), of the peak area of probucol to that of the internal standard.
ing conditions, the relative standard deviation of the ratio of the peak area of probucol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

**Procainamide Hydrochloride**

プロカインアミド塩酸塩

C\textsubscript{13}H\textsubscript{21}N\textsubscript{3}O.HCl: 271.79

4-Amino-N-(2-diethylaminoethyl)benzamide monohydrochloride [614-39-1]

Procainamide Hydrochloride, when dried, contains not less than 98.0% and not more than 101.0% of C\textsubscript{13}H\textsubscript{21}N\textsubscript{3}O.HCl.

**Description**

Procainamide Hydrochloride occurs as a white or light yellow crystalline powder.

It is very soluble in water and soluble in ethanol (99.5).

It is hygroscopic.

**Identification** (1) Determine the infrared absorption spectrum of Procainamide Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Procainamide Hydrochloride (1 in 20) responds to the Qualitative Tests <1.09> for chloride.

**pH** <2.54> Dissolve 1.0 g of Procainamide Hydrochloride in 10 mL of water: the pH of this solution is between 5.0 and 6.5.

**Melting point** <2.60> 165 - 169°C

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Procainamide Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Procainamide Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.17>—Prepare the test solution with 1.0 g of Procainamide Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Procainamide Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 \( \mu \)L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than procainamide from the sample solution is not larger than the peak area of procainamide from the standard solution.

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 \( \mu \)m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.02 mol/L phosphate buffer solution, pH 3.0 and methanol (9:1).

Flow rate: Adjust the flow rate so that the retention time of procainamide is about 9 minutes.

Time span of measurement: About 2 times as long as the retention time of procainamide.

**System suitability**—

Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of procainamide obtained from 10 \( \mu \)L of this solution is equivalent to 40 to 60% of that of procainamide from 10 \( \mu \)L of the standard solution.

System performance: When the procedure is run with 10 \( \mu \)L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of procainamide are not less than 10,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 \( \mu \)L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of procainamide is not more than 2.0%.

**Loss on drying** <2.4> Not more than 0.3% (2 g, 105°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (2 g).

**Assay** Weigh accurately about 0.5 g of Procainamide Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100):7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 27.18 mg of C\textsubscript{13}H\textsubscript{21}N\textsubscript{3}O.HCl

Containers and storage Containers—Tight containers.

**Procainamide Hydrochloride Injection**

プロカインアミド塩酸塩注射液

Procainamide Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of procainamide hydrochloride (C\textsubscript{13}H\textsubscript{21}N\textsubscript{3}O.HCl: 271.79).

**Method of preparation** Prepare as directed under Injections, with Procainamide Hydrochloride.
**Description**  Procainamide Hydrochloride Injection is a clear, colorless or light yellow liquid.

pH: 4.0 – 6.0

**Identification**  (1) To a volume of Procainamide Hydrochloride Injection, equivalent to 10 mg of Procainamide Hydrochloride according to the labeled amount, add 1 mL of dilute hydrochloric acid and water to make 5 mL: the solution responds to the Qualitative Tests <1.09> (1) for primary aromatic amines.

(2) To a volume of Procainamide Hydrochloride Injection, equivalent to 0.1 g of Procainamide Hydrochloride according to the labeled amount, add water to make 100 mL. To 1 mL of this solution add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 277 nm and 281 nm.

(3) Procainamide Hydrochloride Injection responds to the Qualitative Tests <1.09> (2) for chloride.

**Bacterial endotoxins** <4.01>  Less than 0.30 EU/mg.

**Extractable volume** <6.05>  It meets the requirements.

**Foreign insoluble matter** <6.06>  Perform the test according to Method 1: it meets the requirements.

**Insoluble particulate matter** <6.07>  It meets the requirements.

**Sterility** <4.06>  Perform the test according to the Membrane filtration method: it meets the requirements.

**Assay**  Dilute an accurately measured volume of Procainamide Hydrochloride Injection, equivalent to about 0.5 g of procainamide hydrochloride (C₁₃H₂₁N₃O.HCl), with 5 mL of dilute hydrochloric acid and water to make 50 mL, add 10 mL of potassium bromide solution (3 → 10), cool to 15°C or lower, and titrate <2.50> with 0.1 mol/L sodium nitrite VS (potentiometric titration method or amperometric titration).

Each mL of 0.1 mol/L sodium nitrite VS = 27.18 mg of C₁₃H₂₁N₃O.HCl

**Containers and storage**  Containers—Hermetic containers.

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**Procainamide Hydrochloride Tablets**

プロカインアミド塩酸塩錠

Procainamide Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of procainamide hydrochloride (C₁₃H₂₁N₃O.HCl: 271.79).

**Method of preparation**  Prepare as directed under Tablets, with Procainamide Hydrochloride.

**Identification**  To a quantity of powdered Procainamide Hydrochloride Tablets, equivalent to 1.5 g of Procainamide Hydrochloride according to the labeled amount, add 30 mL of water, shake well, filter, and use the filtrate as the sample solution. To 0.2 mL of the sample solution add 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

**Uniformity of dosage units** <6.02>  Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Procainamide Hydrochloride Tablets add 3V/5 mL of 0.02 mol/L phosphate buffer solution, pH 3.0, treat with ultrasonic waves to disintegrate the tablets completely, add 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly V mL so that each mL contains about 2.5 mg of procainamide hydrochloride (C₁₃H₂₁N₃O.HCl), and shake for 5 minutes. Centrifuge this solution, pipet 1 mL of the supernatant liquid, add 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly 250 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of procainamide hydrochloride (C₁₃H₂₁N₃O.HCl) = Mₛ × Aₛ/V × V/20

Mₛ: Amount (mg) of procainamide hydrochloride for assay

**Dissolution** <6.10>  When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Procainamide Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Procainamide Hydrochloride Tablets, withdraw not less than 30 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly V mL so that each mL contains about 7 μg of procainamide hydrochloride (C₁₃H₂₁N₃O.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 0.125 g of procainamide hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 1000 mL. Pipet 5 mL of this solution, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, Aₗ and Aₛ, at 278 nm.

Dissolution rate (%) with respect to the labeled amount of procainamide hydrochloride (C₁₃H₂₁N₃O.HCl) = Mₛ × Aₛ/Aₗ × V/V/V × 1/C × 9/2

Mₛ: Amount (mg) of procainamide hydrochloride for assay

C: Labeled amount (mg) of procainamide hydrochloride (C₁₃H₂₁N₃O.HCl) in 1 tablet

**Assay**  To 10 Procainamide Hydrochloride Tablets add about 300 mL of 0.02 mol/L phosphate buffer solution, pH 3.0, and treat with ultrasonic waves to disintegrate the tablets completely. To this solution add 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly 500 mL, and stir for 5 minutes. Centrifuge this solution, pipet V mL of the supernatant liquid, and add 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly V mL so that each mL contains about 10 μg of procainamide hydrochloride (C₁₃H₂₁N₃O.HCl). Filter this solution through a membrane
filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of procainamide hydrochloride for assay, previously dried at 105°C for 4 hours, divide into 0.2 mol/L phosphate buffer solution, pH 3.0, to make exactly 100 mL. Pipet 2 mL of this solution, add 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of the sample solution. Separately, weigh accurately about 50 mg of procainamide hydrochloride for assay, previously dried at 105°C for 4 hours, dissolve in 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.10> according to the following conditions, and determine the peak areas, A7 and A5, of procainamide in each solution.

\[ M_5 \times \frac{A7}{A5} \times \frac{V'}{V} \times 1/10 \]

**Operating conditions**

- Detector: An ultraviolet absorption photometer (wavelength: 270 nm).
- Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
- Column temperature: A constant temperature of about 40°C.
- Mobile phase: A mixture of 0.02 mol/L phosphate buffer solution, pH 3.0, and methanol (9:1).
- Flow rate: Adjust the flow rate so that the retention time of procainamide is about 9 minutes.

**System suitability**

- System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of procainamide are not less than 10,000 and not more than 1.5, respectively.
- System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of procainamide is not more than 1.0%.

**Containers and storage**

Containers—Tight containers.

### Procaine Hydrochloride

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**Description**

Procaine Hydrochloride occurs as white crystals or crystalline powder.

It is very soluble in water, soluble in ethanol (95), and practically insoluble in diethyl ether.

**Identification**

1. Determine the absorption spectrum of a solution of Procaine Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

2. Determine the infrared absorption spectrum of Procaine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

3. A solution of Procaine Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

**pH**

The pH of a solution prepared by dissolving 1.0 g of Procaine Hydrochloride in 20 mL of water is between 5.0 and 6.0.

**Melting point**

155 – 158°C

**Purity**

1. Clarity and color of solution—Dissolve 1.0 g of Procaine Hydrochloride in 10 mL of water: the solution is clear and colorless.

2. Heavy metals

Proceed with 1.0 g of Procaine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

3. Related substances—To 1.0 g of Procaine Hydrochloride add 5 mL of ethanol (95), dissolve by mixing well, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of 4-aminobenzoic acid in ethanol (95) to make exactly 20 mL, then pipet 1 mL of this solution, add 4 mL of ethanol (95) and water to make exactly 10 mL, and use this solution as the standard solution. Prepare the test with these solutions as directed under Thin-layer Chromatography <2.62>.

Spot 5 μL of each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dibutyl ether, n-hexane and acetic acid (100) (20:4:1) to a distance of about 10 cm, and air-dry the plate. After drying the plate at 105°C for 10 minutes, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. The principal spot from the sample solution stays at the origin.

**Loss on drying**

Not more than 0.5% (1 g, silica gel, 4 hours).

**Residue on ignition**

Not more than 0.1% (1 g).

**Assay**

Weigh accurately about 0.4 g of Procaine Hydrochloride, previously dried, dissolve in 5 mL of hydrochloric acid and 60 mL of water, add 10 mL of a solution of potassium bromide (3 in 10), cool to below 15°C, and titrate <2.50> with 0.1 mol/L sodium nitrite VS (potentiometric titration or amperometric titration).

Each mL of 0.1 mol/L sodium nitrite VS contains 27.28 mg of C13H21N3O.HCl.

**Containers and storage**

Containers—Well-closed contain.
Procarbazine Hydrochloride Injection

プロカルバジン塩酸塩

Procarbazine Hydrochloride Injection is a clear, colorless liquid.

Identification (1) To a volume of Procarbazine Hydrochloride Injection, equivalent to 0.01 g of Procarbazine Hydrochloride according to the labeled amount, add water to make 1000 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 219 nm and 223 nm, and between 289 nm and 293 nm.

(2) Procarbazine Hydrochloride Injection responds to the Qualitative Tests <4.06> (2) for chloride.

pH <2.5> 3.3 - 6.0

Bacterial endotoxins <4.01> Less than 0.02 EU/unit. Apply to the preparations intended for intraspinal administration.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To an exactly measured volume of Procarbazine Hydrochloride Injection, equivalent to about 20 mg of procarbazine hydrochloride (C₁₂H₁₉N₃O.HCl), add the mobile phase to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of procarbazine hydrochloride for assay, previously dried in a desiccator (silica gel) for 4 hours, dissolve in the mobile phase to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography: it meets the requirement.

Amount (mg) of procarbazine hydrochloride

\[ M_s = M_x \times \frac{Q_s}{Q_x} \times \frac{2}{5} \]

Mₜ: Amount (mg) of procarbazine hydrochloride for assay

Internal standard solution—A solution of caffeine in the mobile phase (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 6 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 3.0 with phosphoric acid, and add an amount of sodium 1-pentane sulfonate to make a solution so that containing 0.1%. To 800 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of procarbazine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, procarbazine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of procarbazine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Procarbazine Hydrochloride

プロカルバジン塩酸塩

Procarbazine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of C₁₂H₁₉N₃O.HCl.

Description Procarbazine Hydrochloride occurs as white to light yellowish white crystals or crystalline powder.

It is freely soluble in water, and slightly soluble in ethanol (99.5).

It dissolves in dilute hydrochloric acid.

Melting point: about 223°C (with decomposition).

Identification (1) Dissolve 0.01 g of Procarbazine Hydrochloride in 1 mL of diluted copper (II) sulfate TS (1 in 10), and add 4 drops of sodium hydroxide TS: a green precipitate is formed immediately, and the color changes from green through yellow to orange.
Procarbazine Hydrochloride Hydrate

**Purity** (1) Heavy metals <1.07>—Proceed with 1.0 g of Procarbazine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Procarbazine Hydrochloride in 5.0 mL of a solution of L-cysteine hydrochloride monohydrate in diluted methanol (7 in 10) (1 in 200), and use this solution as the standard solution. Pipet 1 mL of the sample solution, add a solution of L-cysteine hydrochloride monohydrate in diluted methanol (7 in 10) (1 in 200) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Immense slowly, by inclining, a plate of silica gel with fluorescent indicator for thin-layer chromatography in a solution of L-cysteine hydrochloride monohydrate in diluted methanol (7 in 10) (1 in 200), allow to stand for 1 minute, lift the plate from the solution, dry it in cold wind for 10 minutes, then dry in warm wind for 5 minutes, and then dry at 60°C for 5 minutes. After cooling, spot 5 μL each of the sample solution and standard solution on the plate. Develop the plate with a mixture of methanol and ethyl acetate (1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): not more than 1 spot other than the principal spot and the spot of the starting point from the sample solution appears, and is not more intense than the spot from the standard solution.

**Assay** Weigh accurately about 0.15 g of Procarbazine Hydrochloride, previously dried, in a glass-stoppered flask, dissolve in 25 mL of water, add 25 mL of hydrochloric acid, and cool to room temperature. To this solution add 5 mL of chloroform, and titrate <2.50>, while shaking, with 0.05 mol/L potassium iodate VS until the purple color of the chloroform layer disappears. The end point is reached when the red-purple color of the chloroform layer no more reappears within 5 minutes after the purple color disappeared.

Each mL of 0.05 mol/L potassium iodate VS = 8.592 mg of C16H22N2O3.HCl

**Containers and storage** Containers—Tight containers.

Procarbazine Hydrochloride Hydrate contains not less than 98.5% of procarbazine hydrochloride (C16H22N2O3.HCl: 326.82), calculated on the anhydrous basis.

**Description** Procarbazine Hydrochloride Hydrate occurs as white to pale yellowish white crystals or crystalline powder. It is soluble in water, in formic acid and in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Procarbazine Hydrochloride Hydrate (1 in 100) is between 4.0 and 5.0.

It is gradually colored by light.

The solution of Procarbazine Hydrochloride Hydrate (1 in 20) shows no optical rotation.

Melting point: about 195°C (with decomposition).

**Identification** (1) Determine the absorption spectrum of a solution of Procarbazine Hydrochloride Hydrate (7 in 1,000,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Procarbazine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Procarbazine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Containers and storage** Containers—Tight containers.
Neutralization of the hydrochloride with alcoholic potassium hydroxide yields a precipitate of potassium hydrochloride. The precipitate is dissolved in water, adjusted to pH 8, and filtered. The filtrate is treated with 2 mL of titrated hydrochloric acid, and the liberated acetic acid titrated with 0.1 mol/L sodium hydroxide solution. The result is corrected for end-point blank.

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 25 cm in length, packed with octadecl-silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 0.87 g of sodium 1-pentanesulfonate in 1000 mL of water. To 760 mL of this solution add 230 mL of methanol and 10 mL of acetic acid (100).

Flow rate: Adjust the flow rate so that the retention time of procaterol is about 15 minutes.

Selection of column: Dissolve 20 mg each of Procaterol Hydrochloride Hydrate and threoprocaterol hydrochloride in 100 mL of diluted methanol (1 in 2). To 15 mL of this solution add diluted methanol (1 in 2) to make 100 mL. Proceed with 2 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of procaterol and threoprocaterol in this order with the resolution of these peaks being not less than 3.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of procaterol obtained from 2 μL of the standard solution is not less than 3.

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The peaks of procaterol obtained from the sample solution are eluted before those obtained from the standard solution, and the peak area of procaterol from the standard solution is not less than 10 mm.

To 40 mL of 0.1 mol/L perchloric acid VS (potentiometric titration) perform a blank titration. Weigh accurately about 0.25 g of Procaterol Hydrochloride Hydrate, add 2 mL of formic acid, dissolve by warming, and add exactly 15 mL of 0.1 mol/L perchloric acid VS. Add 1 mL of acetic anhydride, heat on a water bath for 30 minutes, cool, add 60 mL of acetic anhydride, and titrate (2.50) the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS = 32.68 mg of C16H22N2O3.HCl

Containers and storage—Containers—Well-closed containers.

Storage—Light-resistant.

Prochlorperazine Maleate

プロクロルベラジンマレイン酸塩

C20H23ClN3S·2C4H4O4: 606.09
2-Chloro-10-[3-(4-methylpiperazin-1-yl)propyl]-10H-phenothiazine dimaleate

[84-02-6]

Prochlorperazine Maleate, when dried, contains not less than 98.0% of C20H23ClN3S·2C4H4O4.

Description Prochlorperazine Maleate occurs as a white to light yellow powder. It is odorless, and has a slightly bitter taste.

It is slightly soluble in acetic acid (100), very slightly soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

It gradually acquires a red tint by light.

Melting point: 195 – 203°C (with decomposition).

Identification (1) Dissolve 5 mg of Prochlorperazine Maleate in 5 mL of sulfuric acid: a red color develops, which darkens slowly on standing. Warm a half of the solution: the color changes to red-purple. To the remainder add 1 drop of potassium dichromate TS: a green-brown color develops, which changes to brown on standing.

(2) Boil 0.5 g of Prochlorperazine Maleate with 10 mL of hydrobromic acid under a reflux condenser for 10 minutes. After cooling, add 100 mL of water, and filter through glass filter (G4). Wash the residue with three 10-mL portions of water, and dry at 105°C for 1 hour: it melts (2.60) between 195°C and 198°C (with decomposition).

(3) Dissolve 0.2 g of Prochlorperazine Maleate in 5 mL of a solution of sodium hydroxide (1 in 10), and extract with three 3-mL portions of diethyl ether [reserve the aqueous layer, and use for test (4)]. Evaporate the combined diethyl ether extracts on a water bath to dryness, dissolve the residue in 10 mL of methanol by warming, and pour into 30 mL of a solution of 2,4,6-trinitrophenol in methanol (1 in 75), previously warmed to 50°C. Allow to stand for 1 hour, collect the crystals, wash with a small amount of methanol, and dry at 105°C for 1 hour: the crystals melt (2.60) between 252°C and 258°C (with decomposition).

(4) To the aqueous layer reserved in (3) add boiling chips, and heat on a water bath for 10 minutes. Cool, add 2 mL of bromine TS, heat on a water bath for 10 minutes, and heat the solution to boil. After cooling, add 2 drops of this solution to 3 mL of a solution of resorcinol in sulfuric acid (1 in 300), and heat on a water bath for 15 minutes: a red-purple color is produced.

Purity Heavy metals (1.07)—Proceed with 1.0 g of Prochlorperazine Maleate according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).
Loss on drying Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition Not more than 0.1% (1 g).

Assay

Weigh accurately about 0.3 g of Prochlorperazine Maleate, previously dried, dissolve in 60 mL of acetic acid (100) while stirring and warming. Cool, and titrate with 0.05 mol/L perchloric acid VS until the color of the solution changes from orange to green (indicator: 0.5 mL of p-naphtholbenzein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS = 15.15 mg of \( \text{C}_{20}\text{H}_{24}\text{ClN}_{3}\text{S.2C}_{4}\text{H}_{4}\text{O}_{4} \)

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Prochlorperazine Maleate Tablets

プロクロルペラジンマレイン酸塩錠

Prochlorperazine Maleate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of prochlorperazine maleate (\( \text{C}_{20}\text{H}_{24}\text{ClN}_{3}\text{S.2C}_{4}\text{H}_{4}\text{O}_{4} \); 606.09).

Method of preparation Prepare as directed under Tablets, with Prochlorperazine Maleate.

Identification (1) Weigh a quantity of powdered Prochlorperazine Maleate Tablets, equivalent to 5 mg of Prochlorperazine Maleate according to the labeled amount, add 15 mL of acetic acid (100), shake, and filter. To 5 mL of the filtrate add 3 mL of sulfuric acid, and shake: a light red color develops. To this solution add 1 drop of potassium permanganate TS: the red color of the test solution is discharged immediately.

Uniformity of dosage units Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Conduct this procedure using light-resistant vessels. To 1 tablet of Prochlorperazine Maleate Tablets add 31/5 mL of a mixture of dilute phosphoric acid (1 in 500) and ethanol (99.5) (1:1), treat with ultrasonic waves until the tablet is disintegrated, and shake vigorously for 10 minutes. Add exactly 1/20 mL of the internal standard solution, and a mixture of dilute phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make 1V mL so that each mL contains about 80 \( \mu \text{g} \) of prochlorperazine maleate (\( \text{C}_{20}\text{H}_{24}\text{ClN}_{3}\text{S.2C}_{4}\text{H}_{4}\text{O}_{4} \)). Centrifuge this solution, and use the supernatant liquid as the sample solution. Proceed as directed in the Assay.

Amount (mg) of prochlorperazine maleate

\[
\text{Amount (mg) of Prochlorperazine Maleate RS} = M_S \times \frac{Q_T}{Q_S} \times \frac{V}{250}
\]

\( M_S \): Amount (mg) of Prochlorperazine Maleate RS

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) (1 in 1000).

Dissolution When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Prochlorperazine Maleate Tablets is not less than 75%.

Start the test with 1 tablet of Prochlorperazine Maleate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 \( \mu \text{m} \). Discard the first 10 mL of the filtrate, pipet \( V' \) mL of the subsequent filtrate, add the dissolution medium to make exactly \( V' \) mL so that each mL contains about 9 \( \mu \text{g} \) of prochlorperazine maleate (\( \text{C}_{20}\text{H}_{24}\text{ClN}_{3}\text{S.2C}_{4}\text{H}_{4}\text{O}_{4} \)) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 18 mg of Prochlorperazine Maleate RS, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry (2.24), using the dissolution medium as the blank, and determine the absorbances, \( A_T \) and \( A_S \), at 255 nm.

Dissolution rate (%) with respect to the labeled amount of prochlorperazine maleate (\( \text{C}_{20}\text{H}_{24}\text{ClN}_{3}\text{S.2C}_{4}\text{H}_{4}\text{O}_{4} \))

\[
= M_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 45
\]

\( M_S \): Amount (mg) of Prochlorperazine Maleate RS

C: Labeled amount (mg) of prochlorperazine maleate (\( \text{C}_{20}\text{H}_{24}\text{ClN}_{3}\text{S.2C}_{4}\text{H}_{4}\text{O}_{4} \)) in 1 tablet

Assay

Conduct this procedure using light-resistant vessels. Weigh accurately the mass of not less than 20 Prochlorperazine Maleate Tablets, and powder in an agate mortar. Weigh accurately a portion of the powder, equivalent to about 8 mg of prochlorperazine maleate (\( \text{C}_{20}\text{H}_{24}\text{ClN}_{3}\text{S.2C}_{4}\text{H}_{4}\text{O}_{4} \)), add 60 mL of a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1), and shake vigorously for 10 minutes.
Add exactly 5 mL of the internal standard solution, and add a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Prochlorperazine Maleate RS, previously dried at 105°C for 3 hours, and dissolve in a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution and a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q<sub>R</sub> and Q<sub>S</sub>, of the peak area of prochlorperazine to that of the internal standard.

\[
M_S = \frac{M_S \times Q_R}{Q_S \times \frac{1}{2}}
\]

\[
Q_S = \frac{M_S \times Q_R}{Q_S \times \frac{1}{2}}
\]

Amount (mg) of prochlorperazine maleate
\[
(C_20H24ClN3S·2C_4H4O_4)
\]

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) (1 in 1000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 257 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2) and acetonitrile (11:9).
Flow rate: Adjust the flow rate so that the retention time of prochlorperazine is about 5 minutes.
System suitability—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, prochlorperazine and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of prochlorperazine to that of the internal standard is not more than 1.0%.

Containers and storage—Containers—Tight containers.
Storage—Light-resistant.

**Progesterone**

プロゲステロン

C<sub>21</sub>H<sub>30</sub>O<sub>2</sub>: 314.46
Pregn-4-ene-3,20-dione [57-83-0]

Progesterone, when dried, contains not less than 97.0% and not more than 103.0% of C<sub>21</sub>H<sub>30</sub>O<sub>2</sub>.

Description
Progesterone occurs as white crystals or crystalline powder.
It is soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Progesterone in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Progesterone RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Progesterone, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Progesterone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Progesterone and Progesterone RS in ethanol (95), respectively, then evaporate the ethanol to dryness, and repeat the test on the residues.

Optical rotation <2.49> [α]<sub>D</sub>: +184° – +194° (after drying, 0.2 g, ethanol (99.5), 10 mL, 100 mm).

Melting point <2.60> 128 – 133°C or 120 – 122°C

Purity Related substances—Dissolve 80 mg of Progesterone in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of diethyl ether and diethylamine (19:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 10 mg each of Progesterone
and Progesterone RS, previously dried, and dissolve each in ethanol (99.5) to make exactly 100 mL. Pipet 5 mL each of these solutions, add ethanol (99.5) to make exactly 50 mL, and use these solution as the sample solution and the standard solution, respectively. Determine the absorbances, $A_F$ and $A_S$, of the sample solution and standard solution at the wavelength of maximum absorption at about 241 nm as directed under Ultraviolet-visible Spectrophotometry $<2.24>$. 

\[
\text{Amount (mg) of progesterone (C$_{21}$H$_{30}$O$_2$) } = M_S \times A_T/A_S
\]

$M_S$: Amount (mg) of Progesterone RS

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

### Progesterone Injection

#### プロゲステロン注射液

Progesterone Injection is an oily solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of progesterone (C$_{21}$H$_{30}$O$_2$: 314.46).

#### Method of preparation Prepare as directed under Injections, with Progesterone.

#### Description Progesterone Injection is a clear, colorless to pale yellow, oily liquid.

#### Identification To 1 mL of Progesterone Injection add 1 mL of diluted ethanol (9 in 10), shake well, take the ethanol layer, shake well with 1 mL of petroleum benzin, and use the ethanol layer as the sample solution. Separately, dissolve about 5 mg of Progesterone RS in 1 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $<2.05>$. Spot 2 $\mu$L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether and diethylamine (19:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, and use this solution as the sample solution. Separately, dissolve about 5 mg of Progesterone RS in 1 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Liquid Chromatography $<2.07>$ according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of progesterone to that of the internal standard.

\[
\text{Amount (mg) of progesterone (C$_{21}$H$_{30}$O$_2$) } = M_S \times Q_T/Q_S \times V/20
\]

$M_S$: Amount (mg) of Progesterone RS

#### Internal standard solution—A solution of testosterone propionate in ethanol (99.5) (1 in 4000).

#### Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 241 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of acetonitrile and water (7:3).

Flow rate: Adjust the flow rate so that the retention time of progesterone is about 6 minutes.

#### System suitability—

System performance: When the procedure is run with 5 $\mu$L of the standard solution under the above operating conditions, progesterone and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 5 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of progesterone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

### Proglumide

#### プログルミド

C$_{18}$H$_{26}$N$_2$O$_4$: 334.41
(4RS)-4-Benzoylamino-$N,N$-dipropylglutaramic acid [6620-60-6]

Proglumide, when dried, contains not less than 98.5% of C$_{18}$H$_{26}$N$_2$O$_4$.

#### Description Proglumide occurs as white crystals or crystalline powder.
It is freely soluble in methanol, soluble in ethanol (95), sparingly soluble in diethyl ether, and very slightly soluble in water.

A solution of Proglumide in methanol (1 in 10) shows no optical rotation.

**Identification (1)** Put 0.5 g of Proglumide in a round bottom tube, add 5 mL of hydrochloric acid, seal the tube, and heat the tube carefully at 120°C for 3 hours. After cooling, open the tube, filter the contents to collect crystals separated out, wash the crystals with 50 mL of cold water, and dry at 100°C for 1 hour: the melting point is between 121°C and 124°C.

(2) Determine the infrared absorption spectrum of Proglumide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Absorbance** <2.24> $E_{1\%}^{1 cm}$ (225 nm): 384 - 414 (after drying, 4 mg, methanol, 250 mL).

**Melting point** <2.60> 148 - 150°C

**Purity (1)** Heavy metals <1.07>—Proceed with 1.0 g of Proglumide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.17>—To 1.0 g of Proglumide add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) and 1.5 mL of hydrogen peroxide (30), burn the sample, and prepare the test solution according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Proglumide in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, ethyl acetate, acetic acid (100) and methanol (50:18:5:4) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.10% (1 g, reduced pressure, phosphorus(V) oxide, 60°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.16 g of Proglumide, previously dried, dissolve in 40 mL of methanol, add 10 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 33.44 mg of C_18H_26N_2O_4

**Containers and storage** Containers—Well-closed containers.

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**1-Proline**

L-プロリン

C_5H_9NO_2: 115.13
(2S)-Pyrrolidine-2-carboxylic acid [147-85-3]

1-Proline contains not less than 99.0% and not more than 101.0% of C_5H_9NO_2, calculated on the dried basis.

**Description** 1-Proline occurs as white crystals or crystalline powder. It has a slightly sweet taste.

It is very soluble in water and in formic acid, and slightly soluble in ethanol (99.5).

It is deliquescent.

**Identification** Determine the infrared absorption spectrum of 1-Proline as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> $[\alpha]_D^20$: $-84.0 - -86.0°$ (1 g, calculated on the dried basis, water, 25 mL, 100 mm).

**pH** <2.54> The pH of a solution of 1.0 g of 1-Proline in 10 mL of water is 5.9 to 6.9.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of 1-Proline in 10 mL of water: the solution is clear and colorless.

(2) Chloride <1.07>—Perform the test with 0.5 g of 1-Proline. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Perform the test with 0.6 g of 1-Proline. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test with 0.25 g of 1-Proline. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metal <1.07>—Proceed with 1.0 g of 1-Proline according to Method 1, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron <1.10>—Prepare the test solution with 1.0 g of 1-Proline according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Weigh accurately about 0.5 g of 1-Proline, and dissolve in 0.5 mL of hydrochloric acid and water to make exactly 100 mL. Pipet 10 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount, equivalent to 2.5 mmol, of L-aspartic acid, L-threonine, L-serine, L-glutamic acid, L-proline, glycine, L-alanine, L-cystine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-lysine hydrochloride, ammonium chloride, L-histidine and L-argi-
nine, dissolve them in 0.1 mol/L hydrochloric acid TS to make exactly 1000 mL, and use this solution as the standard stock solution. Pipet 5 mL of the standard stock solution, and add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 4 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the mass percentage of each amino acid, using the mass of amino acid other than proline in 1 mL of the sample solution obtained from the height of the peaks obtained from the sample and standard solution: the amount of each amino acid other than proline is not more than 0.1%.

**Operating conditions—**

Detector: A visible absorption photometer (wavelength: 570 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography composed with a sulfonated polystyrene (3 μm in particle diameter) (Na type).

Column temperature: A constant temperature of about 57°C.

Chemical reaction vessel temperature: A constant temperature of about 130°C.

Reaction time: About 1 minute.

Mobile phase: Prepare the mobile phases A, B, C, D and E according to the following table, and add 0.1 mL each of caprylic acid.

<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid monohydrate</td>
<td>19.80 g</td>
<td>22.00 g</td>
<td>12.80 g</td>
<td>6.10 g</td>
<td>—</td>
</tr>
<tr>
<td>Trisodium citrate dihydrate</td>
<td>6.19 g</td>
<td>7.74 g</td>
<td>13.31 g</td>
<td>26.67 g</td>
<td>—</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.66 g</td>
<td>7.07 g</td>
<td>3.74 g</td>
<td>54.35 g</td>
<td>—</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>8.00 g</td>
</tr>
<tr>
<td>Ethanol (99.5)</td>
<td>130 mL</td>
<td>20 mL</td>
<td>4 mL</td>
<td>—</td>
<td>100 mL</td>
</tr>
<tr>
<td>Thiodiglycol</td>
<td>5 mL</td>
<td>5 mL</td>
<td>5 mL</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Benzy alcohol</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5 mL</td>
<td>—</td>
</tr>
<tr>
<td>Lauromacrogol solution (1 in 4)</td>
<td>4 mL</td>
<td>4 mL</td>
<td>4 mL</td>
<td>4 mL</td>
<td>4 mL</td>
</tr>
<tr>
<td>Water</td>
<td>a sufficient amount</td>
<td>a sufficient amount</td>
<td>a sufficient amount</td>
<td>a sufficient amount</td>
<td>a sufficient amount</td>
</tr>
</tbody>
</table>

Total amount 1000 mL 1000 mL 1000 mL 1000 mL 1000 mL

Switching of mobile phase: Switch the mobile phases A, B, C, D and E sequentially so that when proceed with 20 μL of the standard solution under the above conditions, aspartic acid, caprylic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, ammonia, histidine and arginine are eluted in this order with the resolution between the peaks of isoleucine and leucine being not less than 1.2.

Reaction reagent: Dissolve 204 g of lithium acetate dihydrate in an appropriate amount of water, add 123 mL of acetic acid (100), 401 mL of 1-methoxy-2-propanol and water to make 1000 mL, pass nitrogen for 10 minutes, and use this solution as Solution (I). Separately, to 979 mL of 1-methoxy-2-propanol add 39 g of ninhydrin, pass nitrogen for 5 minutes, add 81 mg of sodium borohydride, pass nitrogen for 30 minutes, and use this solution as Solution (II). Prepare a mixture with an equal volume of the Solution (I) and (II). (Prepare before use).

Flow rate of mobile phase: 0.20 mL per minute.

Flow rate of reaction reagent: 0.24 mL per minute.

**System suitability—**

System performance: When the test is run with 20 μL of the standard solution under the above operating conditions, the resolution between the peaks of glycine and alanine is not less than 1.2.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak height of each amino acid other than proline in the standard solution is not more than 5.0%, and the relative standard deviation of the retention time is not more than 1.0%.

(8) Residual solvent—Being specified separately.

**Loss on drying <2.41>** Not more than 0.3% (1 g, 105°C, 3 hours).

**Residue on ignition <2.44>** Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.12 g of L-Proline, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 11.51 mg of C₃H₅NO₂

**Containers and storage** Containers—Tight containers.

### Promethazine Hydrochloride

プロメタジン塩酸塩

\[ \text{C}_{17}\text{H}_{20}\text{N}_{2}\text{S}\cdot\text{HCl} : 320.88 \]

\((2RS)-N,N\text{-Dimethyl-1-(10H-phenothiazin-10-yl)propan-2-ylamine monohydrochloride} \]

[58-33-3]

Promethazine Hydrochloride, when dried, contains not less than 98.0% of \(\text{C}_{17}\text{H}_{20}\text{N}_{2}\text{S}\cdot\text{HCl}\).

**Description** Promethazine Hydrochloride occurs as a white to light yellow powder.

It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

It is gradually colored by light.

A solution of Promethazine Hydrochloride (1 in 25) shows on optical rotation.

Melting point: about 223°C (with decomposition).

**Identification** (1) Determine the absorption spectrum of a solution of Promethazine Hydrochloride (1 in 100,000) as
**Propafenone Hydrochloride**

Propafenone Hydrochloride occurs as white crystals or a white crystalline powder. It is freely soluble in formic acid, sparingly soluble in methanol, and slightly soluble in water and in ethanol (99.5). A solution of Propafenone Hydrochloride in methanol (1 in 100) shows no optical rotation.

**Identification**

1. Dissolve 0.1 g of Propafenone Hydrochloride in 20 mL of water by warming. After cooling, to 3 mL of this solution add water to make 500 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

2. Determine the infrared absorption spectrum of Propafenone Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Melting point**

172 – 175°C

**Purity**

1. Heavy metals <1.07>—Proceed with 1.0 g of Propafenone Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

2. Related substances—Dissolve 0.10 g of Propafenone Hydrochloride in 20 mL of the mobile phase in the operating conditions 1 to make exactly 50 mL. Pipet 2 mL of the sample solution, and add the mobile phase in the operating conditions 1 to make exactly 50 mL. Pipet 2.5 mL of this solution, add 2.5 mL of a solution of diphenyl phthalate in methanol (1 in 2000), add the mobile phase in the operating conditions 1 to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions 1 and 2. Determine each
peak area of both solutions by the automatic integration method: the area of each peak other than the peak of propafenone from the sample solution is not larger than the peak area of propafenone from the standard solution.

**Operating conditions 1**
- Detector: An ultraviolet absorption photometer (wavelength: 254 nm)
- Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)
- Column temperature: A constant temperature of about 40°C
- Mobile phase: Dissolve 4.6 g of sodium 1-nonanesulfonate and 2.3 g of phosphoric acid in water to make 1000 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 900 mL of the filtrate add 600 mL of acetonitrile.
- Flow rate: Adjust the flow rate so that the retention time of diphenyl phthalate is about 39 minutes.
- Time span of measurement: Beginning after the solvent peak to the retention time of diphenyl phthalate.

**System suitability 1**
- System performance: Dissolve 12 mg of Propafenone Hydrochloride and 50 mg of isopropyl benzoate in 100 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions 1, propafenone and isopropyl benzoate are eluted in this order with the resolution between these peaks being not less than 5.
- System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions 1, the relative standard deviation of the peak area of propafenone is not more than 2.0%.

**Operating conditions 2**
- Detector, column and column temperature: Proceed as directed in the operation conditions 1.
- Mobile phase: Dissolve 7.33 g of sodium 1-decanesulfonate and 2.3 g of phosphoric acid in water to make 1000 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 700 mL of the filtrate add 700 mL of acetonitrile.
- Flow rate: Adjust the flow rate so that the retention time of diphenyl phthalate is about 11 minutes.
- Time span of measurement: About 2.5 times as long as the retention time of diphenyl phthalate, beginning after the retention time of diphenyl phthalate.

**System suitability 2**
- System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions 2, propafenone and diphenyl phthalate are eluted in this order with the resolution between these peaks being not less than 21.
- System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions 2, the relative standard deviation of the peak area of propafenone is not more than 2.0%.

**Loss on drying**
- Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition**
- Not more than 0.1% (1 g).

**Assay**
Weigh accurately about 0.3 g of Propafenone Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add 50 mL of acetic anhydride, and titrate with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS = 18.90 mg of C₂₁H₂₇NO₃.HCl

**Containers and storage**
Containers—Well-closed containers.

**Propafenone Hydrochloride Tablets**

Propafenone Hydrochloride Tablets contain not less than 96.0% and not more than 104.0% of the labeled amount of propafenone hydrochloride (C₂₁H₂₇NO₃.HCl: 377.90).

**Method of preparation**
Prepare as directed under Tablets, with Propafenone Hydrochloride.

**Identification**
To a quantity of Propafenone Hydrochloride Tablets, equivalent to 0.3 g of Propafenone Hydrochloride according to the labeled amount, add 60 mL of water, and disintegrate by warming. After cooling, centrifuge, and to 3 mL of the supernatant liquid add water to make 500 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>:
- it exhibits maxima between 247 nm and 251 nm, and between 302 nm and 306 nm. Separately, determine the both maximal absorbances, A₁ and A₂, of the solution, the ratio of A₁/A₂ is between 2.30 and 2.55.

**Uniformity of dosage units**
Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Propafenone Hydrochloride Tablets add 30 mL of a mixture of water and acetonitrile (1:1), shake well to disintegrate, add a mixture of water and acetonitrile (1:1) to make exactly 50 mL, and centrifuge. Pipet V mL of the supernatant liquid, equivalent to about 6 mg of propafenone hydrochloride (C₂₁H₂₇NO₃.HCl), add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

**Internal standard solution**—A solution of isopropyl benzoate in methanol (1 in 200).

**Dissolution**
When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Propafenone Hydrochloride Tablets is not less than 75%.

Start the test with 1 tablet of Propafenone Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Dis-
to the first 10 mL of the filtrate, pipet $V$ mL of the subsequent filtrate, add water to make exactly $V'$ mL so that each mL contains about 67 μg of propafenone hydrochloride (C$_{21}$H$_{27}$NO$_3$.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 13 mg of propafenone hydrochloride for assay, previously dried at 105°C for 2 hours, dissolve in water to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, $A_1$ and $A_2$, of the sample solution and standard solution at 305 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of propafenone hydrochloride (C$_{21}$H$_{27}$NO$_3$.HCl)

$$M_5 = \frac{A_1}{A_2} \times \frac{V'}{V} \times \frac{1}{C} \times 450$$

$M_5$: Amount (mg) of propafenone hydrochloride for assay

$C$: Labeled amount (mg) of propafenone hydrochloride (C$_{21}$H$_{27}$NO$_3$.HCl) in 1 tablet

**Assay** To a quantity of Propafenone Hydrochloride Tablets, equivalent to 1.5 g of propafenone hydrochloride (C$_{21}$H$_{27}$NO$_3$.HCl), add 70 mL of a mixture of water and acetonitrile (1:1), shake well to disintegrate, shake well for another 5 minutes, add a mixture of water and acetonitrile (1:1) to make exactly 100 mL, and centrifuge. Pipet 4 mL of the supernatant liquid, and add methanol to make exactly 50 mL. Pipet 5 mL of the solution, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of propafenone hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, $Q_T$ and $Q_s$, of the peak area of propafenone to that of the internal standard.

Amount (mg) of propafenone hydrochloride (C$_{21}$H$_{27}$NO$_3$.HCl)

$$M_5 = \frac{Q_T}{Q_s} \times 50$$

$M_5$: Amount (mg) of propafenone hydrochloride for assay

Internal standard solution—A solution of isopropyl benzoate in methanol (1 in 200).

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 4.6 g of sodium 1-nonanesulfonate and 2.3 g of phosphoric acid in water to make 1000 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 900 mL of the filtrate add 600 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of propafenone is about 8 minutes.

**System suitability—**

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, propafenone and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of propafenone to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

### Propantheline Bromide

プロパンテリン臭化物

**C$_{23}$H$_{30}$BrNO$_3$: 448.39**

N-Methyl-N,N-bis(1-methylethyl)-2-[9H-xanthene-9-ylcarbonyl]oxylethylaminium bromide [50-34-0]

Propantheline Bromide, when dried, contains not less than 98.0% and not more than 102.0% of C$_{23}$H$_{30}$BrNO$_3$.

**Description** Propantheline Bromide occurs as a white to yellowish white, crystalline powder. It is odorless and has a very bitter taste.

It is very soluble in water, in ethanol (95), in acetic acid (100) and in chloroform, soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Propantheline Bromide (1 in 50) is between 5.0 and 6.0.

Melting point: about 161°C (with decomposition, after drying).

**Identification (1)** To 5 mL of a solution of Propantheline Bromide (1 in 20) add 10 mL of sodium hydroxide TS, heat to boil for 2 minutes. Cool to 60°C, and add 5 mL of dilute hydrochloric acid. After cooling, collect the precipitates, and wash with water. Recrystallize from dilute ethanol, and dry at 105°C for 1 hour; the crystals melt at 217°C and 222°C.

(2) Dissolve 0.01 g of the crystals obtained in (1) in 5 mL of sulfuric acid: a vivid yellow to yellow-red color develops.

(3) To 5 mL of a solution of Propantheline Bromide (1 in 10) add 2 mL of dilute nitric acid: this solution responds to the Qualitative Tests <1.09> (1) for bromide.

**Purity** Xanthene-9-carboxylic acid and xanthone—Dissolve 10 mg of Propantheline Bromide in exactly 2 mL of chloroform, and use this solution as the sample solution. Separately, dissolve 1.0 mg of xanthene-9-carboxylic acid and 1.0 mg of xanthone in exactly 40 mL of chloroform, and use this solution as the standard solution. Perform the test immediately with these solutions as directed under Thin-layer Chromatography <2.07>. Spot 25 μL each of the sample solution and standard solution on a plate of silica gel with
fluorescent indicator for thin-layer chromatography, and air-dry the plate for 10 minutes. Develop the plate with a mixture of 1,2-dichloroethane, methanol, water and formic acid (56:24:1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light: the spots from the sample solution corresponding to the spots from the standard solution are not more intense than those from the standard solution.

**Loss on drying** &lt;2.4% Not more than 0.5% (2 g, 105°C, 4 hours).

**Residue on ignition** &lt;2.4% Not more than 0.1% (1 g).

**Assay** Weigh accurately about 1 g of Propantheline Bromide, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 44.84 g of C₂₃H₂₉NO₃.HCl

**Containers and storage** Containers—Well-closed containers.

### Propiverine Hydrochloride

プロピベリン塩酸

![Chemical Structure](image)

C₂₃H₂₉NO₃.HCl: 403.94

1-Methylpiperidin-4-yl 2,2-diphenyl-2-propoxyacetate monohydrochloride [54556-98-8]

Propiverine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.5% of C₂₃H₂₉NO₃.HCl.

**Description** Propiverine Hydrochloride occurs as white crystals or a white crystalline powder.

It is soluble in water and in ethanol (99.5).

**Identification** (1) Dissolve 50 mg of Propiverine Hydrochloride in 20 mL of water, and add acetonitrile to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry &lt;2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Propiverine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Propiverine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry &lt;2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Propiverine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of a solution of Propiverine Hydrochloride (1 in 100) add 6 mL of ethyl acetate, and add 3 drops of silver nitrate TS: a white precipitate is formed, which does not dissolve on the addition of 0.5 mL of dilute nitric acid and shaking. The precipitate dissolves on the addition of 2 mL of ammonia TS and shaking.

**Melting point** 213 – 218°C

**Purity** (1) Sulfate &lt;1.14—Perform the test with 0.40 g of Propiverine Hydrochloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(2) Heavy metals &lt;1.07—Proceed with 1.0 g of Propiverine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 50 mg of Propiverine Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 15 µL each of the sample solution and standard solution as directed under Liquid Chromatography &lt;2.07> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak, having the relative retention time about 0.28 to propiverine, obtained from the sample solution is not larger than 3/10 times the peak area of propiverine from the standard solution, the area of the peak other than propiverine and above mentioned peak from the sample solution is not larger than 1/10 times the peak area of propiverine from the standard solution, and the total area of the peaks other than propiverine from the sample solution is not larger than 1/2 times the peak area of propiverine from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of propiverine, beginning after the solvent peak.

**System suitability**

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of propiverine obtained with 15 µL of this solution is equivalent to 3.5 to 6.5% of that with 15 µL of the standard solution.

System performance: When the procedure is run with 15 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of propiverine are not less than 7000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 15 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propiverine is not more than 2.0%.

(4) Residual solvent—Being specified separately.

**Loss on drying** &lt;2.4% Not more than 1.0% (1 g, 105°C, 1 hour).

**Residue on ignition** &lt;2.4% Not more than 0.1% (1 g).
Assay  Weigh accurately about 50 mg each of Propiverine Hydrochloride and Propiverine Hydrochloride RS, both previously dried, and dissolve each in the mobile phase to make exactly 100 mL. Pipet 10 mL of each of these solutions, add the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.01 > \) according to the following conditions, and determine the peak areas, \( A_T \) and \( A_S \), of propiverine from each solution.

\[
\text{Amount (mg) of propiverine hydrochloride} = \frac{M_S \times A_T}{A_S}
\]

\( M_S \): Amount (mg) of Propiverine Hydrochloride RS

System suitability—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with phenylated silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 2.21 g of potassium dihydrogen phosphate and 1.51 g of sodium 1-octane sulfonate in 650 mL of water, adjust to pH 3.2 with phosphoric acid, and add 350 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of propiverine is about 17 minutes.

System suitability—
System performance: When the procedure is run with 15 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of propiverine are not less than 6000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 15 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propiverine is not more than 1.0%.

Containers and storage  Containers—Tight containers.

Propiverine Hydrochloride Tablets
プロピベリン塩酸塩錠

Propiverine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of propiverine hydrochloride \( (C_{23}H_{29}NO_3 \cdot HCl): 403.94 \).

Method of preparation  Prepare as directed under Tablets, with Propiverine Hydrochloride.

Identification  Shake vigorously a quantity of powdered Propiverine Hydrochloride Tablets, equivalent to 50 mg of Propiverine Hydrochloride according to the labeled amount, with 20 mL of water. Add acetonitrile to make 100 mL, centrifuge, and filter the supernatant liquid, if necessary. Determine the absorption spectrum of the supernatant liquid or the filtrate under Ultraviolet-visible Spectrophotometry \( <2.24 > \); it exhibits a maximum between 257 nm and 261 nm.

Purity  Related substances—Shake vigorously a quantity of powdered Propiverine Hydrochloride Tablets, equivalent to 50 mg of Propiverine Hydrochloride according to the labeled amount, with the mobile phase, add the mobile phase to make exactly 100 mL, centrifuge, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography \( <2.01 > \) according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak, having the relative retention time about 0.28 to propiverine, obtained from the sample solution is not larger than 3/10 times the peak area of propiverine from the standard solution, the area of the peak other than propiverine and the peak mentioned above from the sample solution is not larger than 1/5 times the peak area of propiverine from the standard solution, and the total area of the peaks other than propiverine from the sample solution is not larger than 7/10 times the peak area of propiverine from the standard solution.

Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Propiverine Hydrochloride.
Time span of measurement: About 2.5 times as long as the retention time of propiverine, beginning after the solvent peak.

System suitability—
Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of propiverine obtained with 15 μL of this solution is equivalent to 3.5 to 6.5% of that with 15 μL of the standard solution.
System performance: When the procedure is run with 15 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of propiverine are not less than 7000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 15 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propiverine is not more than 2.0%.

Uniformity of dosage units \( <0.02 > \)  Perform the test according to the following method: it meets the requirement of the Content uniformity test.
To 1 tablet of Propiverine Hydrochloride Tablets add the mobile phase, shake vigorously, add the mobile phase to make exactly 1 mL so that each mL contains about 0.1 mg of propiverine hydrochloride \( (C_{23}H_{29}NO_3 \cdot HCl) \), centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of Propivereine Hydrochloride RS, previously dried at 105°C for 1 hour, and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Propiverine Hydrochloride.

\[
\text{Amount (mg) of propiverine hydrochloride} = \frac{M_S \times A_T}{A_S} \times \frac{V}{500}
\]
$M_S$: Amount (mg) of Propiverine Hydrochloride RS

**Dissolution** 6.10> When the test is performed at 50 revolutions per minute according to Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 20 minutes of Propiverine Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Propiverine Hydrochloride Tablets, withdraw not less than 25 mL of the dissolved solution at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V mL so that each mL contains about 11 μg of propiverine hydrochloride (C$_{23}$H$_{29}$NO$_3$.HCl) according to the labeled amount. Pipet 15 mL of this solution, add exactly 2 mL of 0.1 mol/L hydrochloric acid TS, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Propiverine Hydrochloride Tablets, previously dried at 105°C for 1 hour, and dissolve in the dissolution medium to make exactly 100 mL. Further, pipet 15 mL of this solution, add exactly 2 mL of 0.1 mol/L hydrochloric acid TS, and use this solution as the standard solution. Perform the test with exactly 20 μL of each of the sample solution and standard solution as directed under Dissolution at 105°C for 1 hour, and dissolve in the dissolution medium to make exactly 100 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Propiverine Hydrochloride RS, previously dried at 105°C for 1 hour, and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Propiverine Hydrochloride.

Amount (mg) of propiverine hydrochloride (C$_{23}$H$_{29}$NO$_3$.HCl) = $M_S \times A_T/A_S$

$M_S$: Amount (mg) of Propiverine Hydrochloride RS

**Containers and storage** Container—Tight containers.

**Propranolol Hydrochloride**

プロプラノロール塩酸塩

C$_{16}$H$_{21}$NO$_2$.HCl: 295.80
(2RS)-1-(1-Methylethyl)amino-3-(naphthalen-1-yloxy)propan-2-ol monohydrochloride [318-98-9]

Propranolol Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of C$_{16}$H$_{21}$NO$_2$.HCl.

**Description** Propranolol Hydrochloride occurs as a white, crystalline powder.

It is freely soluble in methanol, soluble in water and in acetic acid (100), and sparingly soluble in ethanol (99.5). A solution of Propranolol Hydrochloride in methanol (1 in 40) shows no optical rotation.

It is gradually colored to yellowish white to light brown by light.

**Identification** (1) Determine the absorption spectrum of a solution of Propranolol Hydrochloride in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry C.2.24, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Propranolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry C.2.25, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Propranolol Hydrochloride (1 in 50) responds to the Qualitative Tests C.1.to (2) for chloride.

pH <2.5> The pH of a solution prepared by dissolving 0.5 g of Propranolol Hydrochloride in 50 mL of water is
Residue on ignition <2.44> Not more than 0.1% (1 g).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Melting point <2.60> 163 – 166°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Propranolol Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Propranolol Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 20 mg of Propranolol Hydrochloride in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL of each of the sample solution and standard solution as directed under Liquid Chromatography <2.03> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than propranolol from the sample solution is not larger than 1/2 times the peak area of propranolol from the standard solution, and the total area of the peaks other than the peak of propranolol is not larger than 2 times the peak area of propranolol from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 292 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecysilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 1.6 g of sodium lauryl sulfate and 0.31 g of tetrabutylammonium phosphate in 450 mL of water, add 1 mL of sulfuric acid and 550 mL of acetonitrile for liquid chromatography, and adjust to pH 3.3 with 2 mol/L sodium hydroxide TS.

Flow rate: Adjust the flow rate so that the retention time of propranolol is about 4 minutes.

Time span of measurement: About 5 times as long as the retention time of propranolol.

System suitability—
Test for required detectability: Measure exactly 5 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of propranolol obtained with 20 μL of this solution is equivalent to 17 to 33% of that with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of propranolol is not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propranolol is not more than 2.0%.

Propranolol Hydrochloride Tablets

Propranolol Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of propranolol hydrochloride (C16H21NO2.HCl: 295.80).

Method of preparation Prepare as directed under Tablets, with Propranolol Hydrochloride.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24> : it exhibits maxima between 288 nm and 292 nm, and between 317 nm and 321 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Propranolol Hydrochloride Tablets add 20 mL of water, and shake until the tablet is completely disintegrated. Add 50 mL of methanol, shake vigorously for 10 minutes, then add methanol to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet V mL of the subsequent filtrate, add methanol to make exactly V mL so that each mL contains about 20 μg of propranolol hydrochloride (C16H21NO2.HCl), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propranolol hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A1 and A2, of the sample solution and standard solution at 290 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>:

\[
\text{Amount (mg) of propranolol hydrochloride (C}16\text{H}_21\text{NO}_2\text{HCl}) = M_S \times A_1 / A_2 \times V / V' \times 1 / 25
\]

M_S: Amount (mg) of propranolol hydrochloride for assay

Assay Weigh accurately about 0.5 g of Propranolol Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100): (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 29.58 mg of C16H21NO2.HCl

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.
specified minute after starting the test, and filter through a
membrane filter with a pore size not exceeding 0.45 \mu m.
Discard the first 10 mL of the filtrate, pipet \( V \) mL of the
subsequent filtrate, add water to make exactly \( V \) mL so that
each mL contains about 10 \mu g of propranolol hydrochloride
\((C_{16}H_{21}NO_2 \cdot HCl)\) according to the labeled amount, and use
this solution as the sample solution. Separately, weigh accurately
about 50 mg of propranolol hydrochloride for assay,
previously dried at 105\(^\circ\)C for 4 hours, and dissolve in water
to make exactly 50 mL. Pipet 1 mL of this solution, add
water to make exactly 100 mL, and use this solution as the
standard solution. Determine the absorbances, \( A_S \) and \( A_T \),
of the sample solution and standard solution at 290 nm as
directed under Ultraviolet-visible Spectrophotometry <2.244>.

Dissolution rate (\%) with respect to the labeled amount
of propranolol hydrochloride \((C_{16}H_{21}NO_2 \cdot HCl)\)
= \( M_S \times A_T / A_S \times V / V \times 1/C \times 18 \)

\( M_S \): Amount (mg) of propranolol hydrochloride for assay

\( C \): Labeled amount (mg) of propranolol hydrochloride
\((C_{16}H_{21}NO_2 \cdot HCl)\) in 1 tablet

Assay
Weigh accurately a portion of the powder, equivalent to about 20
mg of propranolol hydrochloride \((C_{16}H_{21}NO_2 \cdot HCl)\), add 60
mL of methanol, shake for 10 minutes, and add methanol
to make exactly 100 mL. Filter, discard the first 20 mL of the
filtrate, pipet 10 mL of the subsequent filtrate, add methanol
to make exactly 100 mL, and use this solution as the sample
solution. Separately, weigh accurately about 50 mg of
propranolol hydrochloride for assay, previously dried at 105\(^\circ\)C for 4 hours, and dissolve in water
to make exactly 50 mL. Pipet 2 mL of this solution, add methanol
to make exactly 100 mL, and use this solution as the standard
solution. Determine the absorbances, \( A_T \) and \( A_S \), of the sample
solution and standard solution at 290 nm as directed under Ultraviolet-visible Spectrophotometry <2.244>.

Amount (mg) of propranolol hydrochloride
\((C_{16}H_{21}NO_2 \cdot HCl)\)
= \( M_S \times A_T / A_S \times 2/5 \)

\( M_S \): Amount (mg) of propranolol hydrochloride for assay

Containers and storage
Containers—Well-closed containers.

Storage—Light-resistant.

Propyl Parahydroxybenzoate
パラオキシ安息香酸プロピル

\[
\text{C}_{10}\text{H}_{12}\text{O}_3 \colon 180.20
\]
Propyl 4-hydroxybenzoate
[94-13-3]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text
that are not harmonized are marked with symbols (\(* \), \(\dagger \)).

Propyl Parahydroxybenzoate contains not less than
98.0% and not more than 102.0% of \(\text{C}_{10}\text{H}_{12}\text{O}_3\).

\(\dagger\) Description
Propyl Parahydroxybenzoate occurs as colorless crystals or a white, crystalline powder.

It is freely soluble in ethanol (95) and in acetone, and very slightly soluble in water.

Identification
(1) The melting point \(<2.60\dagger\) of Propyl Parahydroxybenzoate is between 96\(^\circ\)C and 99\(^\circ\)C.

(2) Determine the infrared absorption spectrum of Propyl Parahydroxybenzoate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity
(1) Clarity and color of solution—Dissolve 1.0 g of Propyl Parahydroxybenzoate in 10 mL of ethanol (95): the solution is clear and not more intensely colored than the following control solution.

Control solution: To 5.0 mL of Cobalt (II) Chloride CS, 12.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS add water to make 1000 mL.

(2) Acidity—Dissolve 0.2 g of Propyl Parahydroxybenzoate in 5 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mL of 0.1 mol/L sodium hydroxide VS: the solution shows a blue color.

(3) Heavy metals—\(\leq 0.07\dagger\)—Dissolve 1.0 g of Propyl Parahydroxybenzoate in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 25 mL of acetone, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).

(4) Related substances—Dissolve 0.10 g of Propyl Parahydroxybenzoate in 10 mL of acetone, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 \(\mu\)L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, water and acetic acid (100) (70:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained with is not more intense than the spot with the standard solution.

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay
Weigh accurately about 1.0 g of Propyl Parahydroxybenzoate and add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70\(^\circ\)C for 1 hour, and immediately cool in ice. Titrate <2.50> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS = 180.2 mg of \(\text{C}_{10}\text{H}_{12}\text{O}_3\).
Propylene Glycol

Propylene Glycol is a clear, colorless, viscous liquid. It is odorless, and has a slightly bitter taste. It is freely soluble in water, with methanol, with ethanol (95) and with pyridine. It is hygroscopic.

Identification (1) Mix 2 to 3 drops of Propylene Glycol with 0.7 g of triphenylchloromethane, add 1 mL of pyridine, and heat under a reflux condenser on a water bath for 1 hour. After cooling, dissolve the mixture in 20 mL of acetone by warming, shake with 0.02 g of activated charcoal, and filter. Concentrate the filtrate to about 10 mL, and cool. Collect the separated crystals, and dry in a desiccator (silica gel) for 4 hours: the crystals melt between 174°C and 178°C.

(2) Heat gently 1 mL of Propylene Glycol with 0.5 g of potassium hydrogen sulfate: a characteristic odor is evolved.

Specific gravity <2.56 @ 18°C 1.035 – 1.040

Purity (1) Acidity—Mix 10.0 mL of Propylene Glycol with 50 mL of freshly boiled and cooled water, and add 5 drops of phenolphthalein TS and 0.30 mL of 0.1 mol/L sodium hydroxide VS: the solution has a red color.

(2) Chloride <1.07—Perform the test with 2.0 g of Propylene Glycol. Prepare the control solution with 0.40 mL of 0.001 mol/L hydrochloric acid VS (not more than 0.007%).

(3) Sulfate <1.14—Perform the test with 10.0 g of Propylene Glycol. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.002%).

(4) Heavy metals <1.07—Perform the test with 5.0 g of Propylene Glycol according to Method 1. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(5) Arsenic <1.17—Prepare the test solution with 1.0 g of Propylene Glycol according to Method 1, and perform the test (not more than 2 ppm).

(6) Glycerin—Heat 1.0 g of Propylene Glycol with 0.5 g of potassium hydrogen sulfate and evaporate to dryness: no odor of acrolein is perceptible.

Water <2.48 Not more than 0.5% (2 g, volumetric titration, direct titration).

Residue on ignition <2.44 Weigh accurately about 20 g of Propylene Glycol in a tared crucible, and heat to boiling. Stop heating, and immediately ignite to burn. Cool, moisten the residue with 0.2 mL of sulfuric acid, and heat strongly with care to constant mass: the mass of the residue is not more than 0.005%.

Distilling range 2.57 184 – 189°C, not less than 95 vol%.

Propylthiouracil

Propylthiouracil, when dried, contains not less than 98.0% of C\textsubscript{10}H\textsubscript{10}N\textsubscript{2}O\textsubscript{5}S.

Description Propylthiouracil occurs as a white powder. It is odorless, and has a bitter taste. It is sparingly soluble in ethanol (95), and very slightly soluble in water and in diethyl ether.

It dissolves in sodium hydroxide TS and in ammonia TS.

Identification (1) Shake well 0.02 g of Propylthiouracil with 7 mL of bromine TS for 1 minute, and heat until the color of bromine TS disappears. Cool, filter, and add 10 mL of barium hydroxide TS to the filtrate: a white precipitate is produced. The color of the precipitate does not turn purple within 1 minute.

(2) To 5 mL of a hot saturated solution of Propylthiouracil add 2 mL of a solution of sodium pentacyanoammine ferroate (II) n-hydrate (1 in 100): a green color develops.

Melting point 2.60 218 – 221°C

Purity (1) Sulfate <1.14—Triturate Propylthiouracil finely in a mortar. To 0.75 g of the powder add 25 mL of water, heat for 10 minutes on a water bath, cool, filter, and wash the residue with water until the volume of the filtrate becomes 30 mL. To 10 mL of the filtrate add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.077%).

(2) Thiourea—Dissolve 0.30 g of Propylthiouracil in 50 mL of water by heating under a reflux condenser for 5 minutes, cool, and filter. To 10 mL of the filtrate add 3 mL of ammonia TS, shake well, and add 2 mL of silver nitrate TS: the solution has no more color than the following control solution.

Control solution: Weigh exactly 60 mg of thiourea, and dissolve in water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and proceed with 10 mL of this solution in the same manner.

Loss on drying <2.4 Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44 Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Propylthiouracil, previously dried, and add 30 mL of water. Add 30 mL of 0.1
mol/L sodium hydroxide VS from a burette, heat to boil, and dissolve by stirring. Wash down the solid adhering to the wall of the flask with a small amount of water, and add 50 mL of 0.1 mol/L silver nitrate VS with stirring. Boil gently for 5 minutes, add 1 to 2 mL of bromothymol blue TS, and titrate <2.50 with 0.1 mol/L sodium hydroxide VS until a persistent blue-green color develops. Determine the total volume of 0.1 mol/L sodium hydroxide VS consumed.

Each mL of 0.1 mol/L sodium hydroxide VS = 8.512 mg of C₇H₁₀N₂O₅S

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Propylthiouracil Tablets

プロピルチオウラシル錠

Propylthiouracil Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of propylthiouracil (C₇H₁₀N₂O₅S: 170.23).

Method of preparation Prepare as directed under Tablets, with Propylthiouracil.

Identification To a quantity of powdered Propylthiouracil Tablets, equivalent to 0.3 g of Propylthiouracil according to the labeled amount, add 5 mL of ammonia TS, allow to stand for 5 minutes with occasional shaking, add 10 mL of water, and centrifuge. To the supernatant liquid add acetic acid (31), collect the precipitate produced, recrystallize from water, and dry at 105°C for 1 hour: it melts <2.60 between 218°C and 221°C. Proceed with the residue as directed in the Identification under Propylthiouracil.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Propylthiouracil Tablets add 3V/4 mL of 2nd fluid for dissolution test, treat with ultrasonic waves until the tablet is disintegrated, and add 2nd fluid for dissolution test to make exactly V mL so that each mL contains about 0.25 mg of propylthiouracil (C₇H₁₀N₂O₅S). Filter this solution through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 5 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of propylthiouracil (C₇H₁₀N₂O₅S) = Mₛ × A₁/Aₛ × V/V × 1/C × 9

Mₛ: Amount (mg) of propylthiouracil for assay

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Protamine Sulfate

プロタミン硫酸塩

Protamine Sulfate is the sulfate of protamine prepared from the mature sperm of fish belonging to the family Salmonidae.

It has a property to bind with heparin. It binds with not less than 100 Units of heparin per mg, calculated on the dried basis.

Description Protamine Sulfate occurs as a white powder. It is sparingly soluble in water.

Identification (1) Dissolve 1 mg of Protamine Sulfate in 2 mL of water, add 5 drops of a solution prepared by dissolving 0.1 g of 1-naphthol in 100 mL of diluted ethanol (7 in 10)
and 5 drops of sodium hypochlorite TS, then add sodium hydroxide TS until the solution becomes alkaline: a vivid red color develops.

(2) Dissolve 5 mg of Protamine Sulfate in 1 mL of water by warming, add 1 drop of a solution of sodium hydroxide (1 in 10) and 2 drops of copper (II) sulfate TS: a red-purple color develops.

(3) An aqueous solution of Protamine Sulfate (1 in 20) responds to the Qualitative Tests <1.09> for sulfate.

**pH** &lt;2.54&gt; Dissolve 1.0 g of Protamine Sulfate in 100 mL of water: the pH of this solution is between 6.5 and 7.5.

**Purity (1)** Clarity and color of solution—Dissolve 0.10 g of Protamine Sulfate in 10 mL of water: the solution is clear and colorless.

(2) Absorbance—Dissolve 0.10 g of Protamine Sulfate in 10 mL of water, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry &lt;2.24&gt;: the absorbance between 260 nm and 280 nm is not more than 0.1.

**Loss on drying** &lt;2.41&gt; Not more than 5.0% (1 g, 105°C, 3 hours).

**Nitrogen content** Weigh accurately about 10 mg of Protamine Sulfate, and perform the test as directed under Nitrogen Determination &lt;1.08&gt;: the amount of nitrogen (N:14.01) is 22.5 – 25.5%, calculated on the dried basis.

**Heparin-binding capacity**

(i) Sample solution (a)—Weigh accurately about 15 mg of Protamine Sulfate, and dissolve in water to make exactly 100 mL. Repeat this procedure 3 times, and use the solutions so obtained as the sample solutions (a₁), (a₂) and (a₃).

(ii) Sample solution (b)—Pipet 10 mL each of the sample solutions (a₁), (a₂) and (a₃), add exactly 5 mL of water to them, and use these solutions as the sample solutions (b₁), (b₂) and (b₃).

(iii) Sample solution (c)—Pipet 10 mL each of the sample solutions (a₁), (a₂) and (a₃), add exactly 20 mL of water to them, and use these solutions as the sample solutions (c₁), (c₂) and (c₃).

(iv) Standard solution—Dissolve Heparin Sodium RS in water to make a solution containing exactly about 20 Units per mL.

(v) Procedure—Transfer exactly 2 mL of the sample solution to a cell for spectrophotometer, add the standard solution dropwise while mixing, and determine the transmission at 500 nm as directed under Ultraviolet-visible Spectrophotometry &lt;2.24&gt;: Continue the addition until a sharp change in the transmittance is observed, and note the volume, V mL, of the standard solution added. Repeat this procedure 2 times for each sample solution.

(vi) Calculation—Calculate the amount of heparin bound with 1 mg of the sample by the following formula from the volume of titrant on each sample solution, and calculate the average of 18 results obtained. The assay is not valid unless each relative standard deviation of 6 results obtained from the sample solution (a), sample solution (b) and sample solution (c) is not more than 5%, respectively, and also unless each relative standard deviation of 6 results obtained from 3 sets, (a₁, b₁, c₁), (a₂, b₂, c₂) and (a₃, b₃, c₃) is not more than 5%, respectively.

Amount (heparin Unit) of heparin bound to 1 mg of Protamine Sulfate

\[
S \times V \times 50/M_f \times d
\]

**Sulfate content** Weigh accurately about 0.15 g of Protamine Sulfate, dissolve in 75 mL of water, add 5 mL of 3 mol/L hydrochloric acid TS, and heat to boil. Add gradually 10 mL of barium chloride TS while boiling, and allow to stand for 1 hour while heating. Filter the precipitate formed, wash the precipitate with warm water several times, and transfer the precipitate into a tared crucible. Dry the precipitate, and incinerate by ignition to constant mass: the amount of sulfate (SO₄) is 16 – 22%, calculated on the dried basis, where 1 g of the residue is equivalent to 0.4117 g of SO₄.

**Containers and storage** Containers—Tight containers.

### Protamine Sulfate Injection

プロタミン硫酸塩注射液

Protamine Sulfate Injection is an aqueous solution for injection.

It contains not less than 92.0% and not more than 108.0% of the labeled amount of Protamine Sulfate. It binds with not less than 100 Units of heparin per mg of the labeled amount.

**Method of preparation** Prepare as directed under Injections, with Protamine Sulfate.

**Description** Protamine Sulfate Injection is a colorless liquid. It is odorless or has the odor of preservatives.

**Identification (1)** Dilute a volume of Protamine Sulfate Injection, equivalent to 1 mg of Protamine Sulfate according to the labeled amount, with water to make 2 mL, and proceed as directed in the Identification (1) under Protamine Sulfate.

(2) Dilute a volume of Protamine Sulfate Injection, equivalent to 5 mg of Protamine Sulfate according to the labeled amount, with water to make 1 mL, and proceed as directed in the Identification (2) under Protamine Sulfate.

**pH** &lt;2.54&gt; 5.0 – 7.0

**Bacterial endotoxins** &lt;4.01&gt; Less than 6.0 EU/mg.

**Extractable volume** &lt;6.05&gt; It meets the requirement.

**Foreign insoluble matter** &lt;6.06&gt; Perform the test according to Method 1: it meets the requirement.

**Insoluble particulate matter** &lt;6.07&gt; It meets the requirement.

**Sterility** &lt;4.06&gt; Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay (1)** Protein—Pipet a volume of Protamine Sulfate Injection, equivalent to about 10 mg of Protamine Sulfate,
Prothionamide

![Structure of Prothionamide](image)

C₉H₁₂N₂S: 180.27
2-Propylpyridine-4-carbothioamide

[14222-60-7]

Prothionamide, when dried, contains not less than 98.0.0% of C₉H₁₂N₂S.

**Description** Prothionamide occurs as yellow crystals or crystalline powder. It has a slight, characteristic odor. It is freely soluble in methanol and in acetic acid (100), soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water. It dissolves in dilute hydrochloric acid and in dilute sulfuric acid.

**Identification (1)** Mix 0.05 g of Prothionamide with 0.1 g of 1-chloro-2,4-dinitrobenzene, transfer about 10 mg of this mixture to a test tube, and heat for several seconds over a small flame until the mixture is fused. Cool, and add 3 mL of potassium hydroxide-ethanol TS: a red to orange-red color develops.

(2) Place 0.5 g of Prothionamide in a 100-mL beaker, and dissolve in 20 mL of sodium hydroxide TS by heating while shaking occasionally: the gas evolved turns a moistened red litmus paper to blue. Boil gently, and evaporate the solution to 3 to 5 mL. After cooling, add gradually 20 mL of acetic acid (100), and heat on a water bath: the gas evolved darkens moistened lead (II) acetate paper. Evaporate the solution on a water bath to 3 to 5 mL with the aid of a current of air, cool, add 10 mL of water, and mix well. Filter the crystals by suction, recrystallize from water immediately, and dry in a desiccator (in vacuum, silica gel) for 6 hours: the crystals melt between 198°C and 203°C (with decomposition).

**Melting point** 142 - 145°C

**Purity (1)** Clarity and color of solution—Dissolve 0.5 g of Prothionamide in 20 mL of ethanol (95): the solution is clear, and shows a yellow color.

(2) Acidity—Dissolve 3.0 g of Prothionamide in 20 mL of methanol with warming. Add 100 mL of water to the solution, cool in an ice water bath with agitation, and remove any precipitate by filtration. Allow 80 mL of the filtrate to cool to room temperature, and add 0.8 mL of cresol red TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Heavy metals ≤1.0 ppm—Proceed with 1.0 g of Prothionamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic ≤1.1 ppm—Prepare the test solution with 0.6 g of Prothionamide according to Method 3, and perform the test. To the test solution add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and ignite to burn (not more than 3.3 ppm).

**Loss on drying** Not more than 0.5% (1 g, 80°C, 3 hours).

**Residue on ignition** Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Prothionamide, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ≤2.5% with 0.1 mol/L perchloric acid VS until the color of the solution changes from orange-red to dark orange-brown (indicator: 2 mL of p-naphtholbenzein TS). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS = 18.03 mg of C₉H₁₂N₂S

**Containers and storage** Containers—Hermetic containers.

Protirelin

![Structure of Protirelin](image)

C₁₆H₂₂N₆O₄: 362.38
5-Oxo-L-prolyl-L-histidyl-L-prolinamide

[24305-27-9]

Protirelin contains not less than 98.5% of C₁₆H₂₂N₆O₄, calculated on the dehydrated basis.

**Description** Protirelin occurs as a white powder. It is freely soluble in water, in methanol, in ethanol (95) and in acetic acid (100). It is hygroscopic.

**Identification (1)** Take 0.01 g of Protirelin in a test tube made of hard glass, add 0.5 mL of 6 mol/L hydrochloric acid TS, seal the upper part of the tube, and heat carefully at...
110°C for 5 hours. After cooling, open the seal, transfer the contents into a beaker, and evaporate on a water bath to dryness. Dissolve the residue in 1 mL of water, and use this solution as the sample solution. Separately, dissolve 0.08 g of L-glutamic acid, 0.12 g of L-histidine hydrochloride monohydrate and 0.06 g of L-proline in 20 mL of water, and use these solutions as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50 mL of acetic acid (100), and titrate with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS = 7.248 mg of C_{16}H_{22}N_{6}O_{4}

Containers and storage Containers—Tight containers.

**Protirelin Tartrate Hydrate**

![Chemical Structure](image)

Protirelin Tartrate Hydrate, calculated on the anhydrous basis, contains not less than 98.5% of protirelin tartrate (C_{16}H_{22}N_{6}O_{4}.C_{4}H_{6}O_{6}.H_{2}O: 512.48). It is freely soluble in water, sparingly soluble in acetic acid (100), and practically insoluble in ethanol (95) and in diethyl ether.

Dissolve 0.20 g of Protirelin Tartrate Hydrate in 10 mL of water: the solution is clear and colorless. The pH of this solution is between 7.5 and 8.5.

### Heavy metals

Proceed with 0.1 g of Protirelin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

### Related substances

Dissolve 0.20 g of Protirelin in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spray evenly a mixture of a solution of sulfanilic acid in 1 mol/L hydrochloric acid TS, and boil for 7 hours under a reflux condenser. After cooling, evaporate 2.0 mL of this solution on a water bath to dryness, dissolve the residue in 6 mol/L hydrochloric acid TS, and boil for 7 hours under a reflux condenser. Use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Develop the plate with a mixture of 1-butanol, water, acetic acid (100) and pyridine (4:1:1:1) to a distance of about 12 cm, and dry the plate at 100°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the three spots obtained from the sample solution are not more intense than the spot from the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Develop the plate with a mixture of 1-butanol, water, acetic acid (100) and pyridine (4:1:1:1) to a distance of about 12 cm, and dry the plate at 100°C for 30 minutes. Spray evenly a mixture of a solution of sulfanilic acid in 1 mol/L hydrochloric acid TS by heating, and use this solution as the sample solution. Separately, dissolve 22 mg of L-glutamic acid, 32 mg of L-histidine hydrochloride monohydrate and 17 mg of L-proline in 2.0 mL of 0.1 mol/L hydrochloric acid TS by heating, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Develop the plate with a mixture of 1-butanol, water, acetic acid (100) and pyridine (4:1:1:1) to a distance of about 12 cm, and dry at 100°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and dry at 80°C for 5 minutes: the three spots obtained from the sample solution show, respectively, the same color and the same Rf value as the corresponding spots from the standard solution.
(4) A solution of Protirelin Tartrate Hydrate (1 in 40) responds to the Qualitative Tests <1.09> for tartrate.

**Optical rotation** <2.49> [α]D: −50.0° to −53.0° (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

**pH** <2.54> Dissolve 1.0 g of Protirelin Tartrate Hydrate in 100 mL of water: the pH of this solution is between 3.0 and 4.0.

**Purity (1)** Clarity and color of solution—Dissolve 0.10 g of Protirelin Tartrate Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Protirelin Tartrate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Take 1.0 g of Protirelin Tartrate Hydrate in a porcelain crucible. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), ignite the ethanol, and heat gradually to incinerate. If a carbonized material still remains in this method, moisten with a small quantity of nitric acid, and ignite to incinerate. After cooling, add 10 mL of dilute hydrochloric acid, heat on a water bath to dissolve the residue, use this solution as the test solution, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.60 g of Protirelin Tartrate Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.63>. Spot 5 μL each of the sample solution and standard solution on a plate (1) of silica gel for thin-layer chromatography. Spot 5 μL of the sample solution on a plate (2) of silica gel for thin-layer chromatography. Develop the plates with a mixture of chloroform, methanol and ammonia solution (28) (6:4:1) to a distance of about 10 cm, and dry at 100°C for 30 minutes. Spray evenly a mixture of a solution of sulfanilic acid in 1 mol/L hydrochloric acid TS (1 in 200) and a solution of sodium nitrite (1 in 20) (1:1) on the plate (1), and air-dry the plate. Then, spray evenly a solution of sodium carbonate decahydrate (1 in 10) on the plate: the spots other than the principal spot from the sample solution are not more intense than those from the standard solution in color. On the other hand, spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate (2), and dry at 80°C for 5 minutes: no colored spot is obtained.

**Water** <2.48> Not more than 4.5% (0.2 g, volumetric titration, direct titration).

**Residue on ignition** <2.44> Not more than 0.2% (0.5 g).

**Assay** Weigh accurately about 0.5 g of Protirelin Tartrate Hydrate, dissolve in 80 mL of acetic acid (100) by warming, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 51.25 mg of C₆H₅N₂O₄.C₂H₃NO₄

Containers and storage Containers—Well-closed containers.

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**Pullulan**

Pullulan is a neutral simple polysaccharide produced by the growth of *Aureobasidium pullulans*. It has a chain structure of repeated α-1,6 binding of maltotriose composed of three glucose in α-1,4 binding.

**Description** Pullulan occurs as a white powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

**Identification (1)** Dissolve 10 g of Pullulan in 100 mL of water with stirring by adding in small portions: a viscous solution is produced.

(2) Mix 10 mL of the viscous solution obtained in (1) with 0.1 mL of pullulanase TS, and allow to stand: the solution loses its viscosity.

(3) To 10 mL of a solution of Pullulan (1 in 50) add 2 mL of macrogol 600: a white precipitate is formed immediately.

**Viscosity** <2.53> Take exactly 10.0 g of Pullulan, previously dried, dissolve in water to make exactly 100 g, and perform the test at 30 ± 0.1°C as directed in Method 1: the kinematic viscosity is between 100 and 180 mm²/s.

**pH** <2.54> Dissolve 1.0 g of Pullulan in 10 mL of freshly boiled and cooled water: the pH is between 4.5 and 6.5.

**Purity (1)** Heavy metals <1.07>—Proceed with 4.0 g of Pullulan according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 5 ppm).

(2) Nitrogen—Weigh accurately about 3 g of Pullulan, previously dried, and perform the test as directed under Nitrogen Determination <1.08>: the amount of nitrogen (N: 14.01) is not more than 0.05%. Use 12 mL of sulfuric acid for the decomposition, and add 40 mL of a solution of sodium hydroxide (2 in 5).

**Description** Polysaccharides—Dissolve 0.8 g of Pullulan, previously dried, in 100 mL of water, and designate this solution as the sample stock solution. To 1 mL of the sample stock solution add 0.1 mL of potassium chloride saturated solution, and shake vigorously with 3 mL of methanol. Centrifuge, and use the supernatant liquid as the sample solution. Separately, pipet 1 mL of the sample stock solution, add water to make exactly 50 mL, and use this solution as the standard solution. Pipet 0.2 mL each of the sample solution, the standard solution and water, transfer them gently to each test tube containing 5 mL of a solution
of anthrone in diluted sulfuric acid (3 in 4) (1 in 500) and cooling in ice water, stir immediately, then heat at 90°C for 10 minutes, and cool immediately. Perform the test with these solutions so obtained as directed under Ultraviolet-visible Spectrophotometry 2.24, using water as a blank, and determine the absorbances at 620 nm, \( A_T \), \( A_S \) and \( A_B \); the amount of monosaccharide and oligosaccharides is not more than 10.0%.

Amount (% of monosaccharide and oligosaccharides

\[
= \frac{(A_T - A_B)}{(A_S - A_B)} \times 8.2
\]

Loss on drying <2.4> Not more than 6.0% (1 g, in vacuum, 90°C, 6 hours).

Residue on ignition <2.4> Not more than 0.3% (2 g).

Containers and storage Containers—Well-closed containers.

**Pyrantel Pamoate**

ピランテルパモ酸塩

\[
C_{11}H_{14}N_2S \cdot C_{23}H_{16}O_6 \cdot 594.68
\]

1-Methyl-2-[(1E)-2-(thien-2-yl)vinyl]-1,4,5,6-tetrahydropyrimidine mono-[4,4'-methylenebis(3-hydroxy-2-naphthoate)] (1/1)

[22204-24-6]

Pyrantel Pamoate, when dried, contains not less than 98.0% of \( C_{11}H_{14}N_2S \cdot C_{23}H_{16}O_6 \).

**Description** Pyrantel Pamoate occurs as a light yellow to yellow, crystalline powder. It is odorless and tasteless.

It is sparingly soluble in \( N,N \)-dimethylformamide, very slightly soluble in methanol and ethanol (95), and practically insoluble in water, in ethyl acetate and in diethyl ether.

Melting point: 256 – 264°C (with decomposition).

**Identification** (1) To 0.05 g of Pyrantel Pamoate add 10 mL of methanol and 1 mL of a mixture of hydrochloric acid and methanol (1:1), and shake vigorously: a yellow precipitate is produced. Filter the solution, and use the filtrate as the sample solution. Use the precipitate for the test (2). To 0.5 mL of the sample solution add 1 mL of a solution of 2,3-indolinedione in sulfuric acid (1 in 1000): a red color develops.

(2) Collect the precipitate obtained in the test (1), wash with methanol, and dry at 105°C for 1 hour. To 0.01 g of the dried precipitate add 10 mL of methanol, shake well, and filter. To 5 mL of the filtrate add 1 drop of iron (III) chloride TS: a green color develops.

(3) Dissolve 0.1 g of Pyrantel Pamoate in 50 mL of \( N,N \)-dimethylformamide, and add methanol to make 200 mL. To 2 mL of the solution add a solution of hydrochloric acid in methanol (9 in 1000) to make 100 mL. Determine the absorbance spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry 2.24, and compare the spectrum with the Reference Spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Pyrantel Pamoate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry 2.25, and compare the spectrum with the Reference Spectrums: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** (1) Chloride <1.07>—To 1.0 g of Pyrantel Pamoate add 10 mL of dilute nitric acid and 40 mL of water, and heat on a water bath with shaking for 5 minutes. After cooling, add water to make 50 mL, and filter. To 20 mL of the filtrate add 2 mL of dilute nitric acid and water to make 50 mL. Proceed the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Sulfate <1.14>—To 0.75 g of Pyrantel Pamoate add 5 mL of dilute hydrochloric acid and water to make 100 mL, and heat on a water bath for 5 minutes with shaking. After cooling, add water to make 100 mL, and filter. To 20 mL of the filtrate add water to make 50 mL. Proceed the test using this solution as the test solution. Prepare the control solution with 0.45 mL of 0.005 mol/L sulfurous acid VS (not more than 0.144%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Pyrantel Pamoate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Pyrantel Pamoate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—The procedure should be performed under protection from direct sunlight in light-resistant vessels. Dissolve 0.10 g of Pyrantel Pamoate in 10 mL of \( N,N \)-dimethylformamide, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add \( N,N \)-dimethylformamide to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography 2.05. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the spot of pyrantel and the spot of pamoic acid from the sample solution are not more intense than the spot of pyrantel (RF value: about 0.3) from the standard solution.

**Loss on drying** <2.4> Not more than 1.0% (1 g, 105°C, 2 hours).

**Residue on ignition** <2.4> Not more than 0.3% (1 g).

**Assay** Weigh accurately about 0.5 g of Pyrantel Pamoate, previously dried, add 25 mL of chloroform and 25 mL of sodium hydroxide TS, shake for 15 minutes, and extract. Extract further with two 25-mL portions of chloroform. Filter each extract through 5 g of anhydrous sodium sulfate on a pledget of absorbent cotton. Combine the chloroform extracts, add 30 mL of acetic acid (100), and titrate <2.59> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal
Pyrazinamide / Official Monographs

Pyrazinamide

Pyrazinamide, when dried, contains not less than 99.0% and not more than 101.0% of C₅H₅N₃O.

Description  Pyrazinamide occurs as white crystals or crystalline powder.

It is sparingly soluble in water and in methanol, and slightly soluble in ethanol (99.5) and in acetic anhydride.

Identification (1) Determine the absorption spectrum of a solution of Pyrazinamide in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pyrazinamide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 188 – 193°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Pyrazinamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Pyrazinamide in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.60>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100): (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay  Weigh accurately about 0.1 g of Pyrazinamide, previously dried, dissolve in 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 12.31 mg of C₃H₅N₃O

Containers and storage  Containers—Tight containers.

Pyridostigmine Bromide

Pyridostigmine Bromide occurs as a white, crystalline powder. It is odorless or has a slightly characteristic odor.

It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

The pH of a solution of Pyridostigmine Bromide (1 in 10) is between 4.0 and 6.0.

It is deliquescent.

Identification (1) Dissolve 0.02 g of Pyridostigmine Bromide in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the absorption spectrum of a solution of Pyridostigmine Bromide in 10 mL of water, add 5 mL of Reinecke salt TS: a light red precipitate is produced.

(3) To 0.1 g of Pyridostigmine Bromide add 0.6 mL of sodium hydroxide TS: the unpleasant odor of dimethylamine is perceptible.

(4) A solution of Pyridostigmine Bromide (1 in 50) responds to the Qualitative Tests <1.09> for Bromide.

Melting point <2.60> 153 – 157°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Pyridostigmine Bromide in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Pyridostigmine Bromide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g
of Pyridostigmine Bromide according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Pyridostigmine Bromide in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add ethanol (95) to make exactly 10 mL. Pipet 1 mL of this solution, add ethanol (95) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.63). Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform and ammonium chloride TS (5:4:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution in color.

**Loss on drying** (2.41) Not more than 2.0% (1 g, in vacuum, phosphorus (V) oxide, 100°C, 5 hours).

**Residue on ignition** (2.44) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.2 g of Pyridostigmine Bromide, previously dried, dissolve in 10 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

\[
\text{Each mL of 0.1 mol/L perchloric acid VS} = 26.11 \text{ mg of C}_5\text{H}_3\text{BrN}_2\text{O}_2
\]

**Containers and storage** Containers—Hermetic containers.

### Pyridoxine Hydrochloride

#### Vitamin B₆

ピリドキシン塩酸塩

C₉H₁₇NO₃.HCl: 205.64
4,5-Bis(hydroxymethyl)-2-methylpyridin-3-ol monohydrochloride [58-56-0]

Pyridoxine Hydrochloride, when dried, contains not less than 98.0% and not more than 101.0% of C₉H₁₇NO₃.HCl.

**Description** Pyridoxine Hydrochloride occurs as a white to pale yellow, crystalline powder.

It is freely soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in acetic anhydride and in acetic acid (100).

It is gradually affected by light.

Melting point: about 206°C (with decomposition).

**Identification** (1) Determine the absorption spectrum of a solution of Pyridoxine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry (2.24), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pyridoxine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pyridoxine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry (2.25), and compare the spectrum with the Reference Spectrum or the spectrum of Pyridoxine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Pyridoxine Hydrochloride (1 in 10) responds to the Qualitative Tests (1.09) for chloride.

**pH** (2.54) The pH of a solution prepared by dissolving 1.0 g of Pyridoxine hydrochloride in 20 mL of water is between 2.5 and 3.5.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Pyridoxine hydrochloride in 50 mL of water is between 2.5 and 3.5.

(2) Heavy metals (1.07)—Proceed with 1.0 g of Pyridoxine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Related substances—Dissolve 1.0 g of Pyridoxine Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 2.5 mL of the sample solution, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography (2.63). Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and air-dry the plate. Develop the plate with a mixture of acetone, tetrahydrofuran, hexane and ammonia solution (28) (65:13:13:9) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of sodium carbonate in diluted ethanol (3 in 10) (1 in 20) on the plate. After air-drying, spray evenly a solution of 2,6-dibromo-N-chloro-1,4-benzoquinone monoxide in ethanol (99.5) (1 in 1000) on the plate, and air-dry: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**Loss on drying** (2.41) Not more than 0.30% (1 g, in vacuum, silica gel, 4 hours).

**Residue on ignition** (2.44) Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.2 g of Pyridoxine Hydrochloride, previously dried, add 5 mL of acetic acid (100) and 5 mL of acetic anhydride, dissolve by gentle boiling, cool, add 30 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

\[
\text{Each mL of 0.1 mol/L perchloric acid VS} = 20.56 \text{ mg of C}_5\text{H}_3\text{NO}_3\text{HCl}
\]

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.
Pyridoxine Hydrochloride Injection

Vitamin B₆ Injection

ピリドキシン塩酸塩注射液

Pyridoxine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of pyridoxine hydrochloride (C₈H₁₁NO₃.HCl: 205.64).

Method of preparation Prepare as directed under Injections, with Pyridoxine Hydrochloride.

Description Pyridoxine Hydrochloride Injection is a colorless or pale yellow, clear liquid.

pH: 3.0 – 6.0

Identification (1) To a volume of Pyridoxine Hydrochloride Injection, equivalent to 0.05 g of Pyridoxine Hydrochloride according to the labeled amount, add 0.1 mol/L hydrochloric acid TS to make 100 mL. To 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible spectrophotometry (2.24): it exhibits a maximum between 288 nm and 292 nm.

(2) To a volume of Pyridoxine Hydrochloride Injection, equivalent to 0.01 g of Pyridoxine Hydrochloride according to the labeled amount, add water to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Pyridoxine Hydrochloride RS, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 200 mL, and use this solution as the sample solution. Separately, dissolve to the labeled amount, add water to make 10 mL, and use this solution as the standard solution. Perform the test according to the Membrane filtration method: it meets the requirement.

Bacterial endotoxins <4.01> Less than 3.0 EU/mg.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Sterility test: it meets the requirement.

Assay Measure exactly a volume of Pyridoxine Hydrochloride Injection, equivalent to about 20 mg of pyridoxine hydrochloride (C₈H₁₁NO₃.HCl), dilute with water, if necessary, and add water to make exactly 100 mL. Pipet 25 mL of this solution, add water to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Pyridoxine Hydrochloride RS, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test according to the Membrane filtration method: it meets the requirement.

Pyroxylin

ピロキシリン

Pyroxylin is a nitric acid ester of cellulose. It is usually moistened with 2-propanol or some other solvent.

Description Pyroxylin occurs as a white cotton-like substance or white flakes.

It is freely soluble in acetone, and very slightly soluble in diethyl ether.

Upon heating or exposure to light, it is decomposed with the evolution of nitrous acid vapors.

Identification Ignite Pyroxylin: it burns very rapidly with a luminous flame.

Purity (1) Clarity of solution—Dissolve 1.0 g of Pyroxylin, previously dried at 105°C for 2 hours, in 25 mL of a mixture of diethyl ether and ethanol (95) (3:1): the solution is clear.

(2) Acidity—Shake 1.0 g of Pyroxylin, previously dried at 80°C for 2 hours, with 20 mL of water for 10 minutes: the filtrate is neutral.

(3) Water-soluble substances—Evaporate 10 mL of the filtrate obtained in (2) on a water bath to dryness, and dry at 105°C for 1 hour: the mass of the residue is not more than 1.5 mg.

(4) Residue on ignition—Weigh accurately about 2 g of Pyroxylin, previously dried at 80°C for 2 hours, and moisten with 10 mL of a solution of castor oil in acetone (1 in 20) to gelatinize the sample. Ignite the contents to carbonize the sample, heat strongly at about 500°C for 2 hours, and allow to cool in a desiccator (silica gel): the amount of the residue is not more than 0.30%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, packed loosely, remote from
fire, and preferably in a cold place.

**Pyrrolnitrin**

ピロールニトリン

\[ \text{C}_{10} \text{H}_{6} \text{Cl}_2 \text{N}_2 \text{O}_2: 257.07 \]

3-Chloro-4-(3-chloro-2-nitrophenyl)pyrrole

[1018-71-9]

Pyrrolnitrin contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dried basis. The potency of Pyrrolnitrin is expressed as mass (potency) of pyrrolnitrin (\( \text{C}_{10} \text{H}_{6} \text{Cl}_2 \text{N}_2 \text{O}_2 \)).

**Description**

Pyrrolnitrin occurs as yellow to yellow-brown, crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (95), and practically insoluble in water.

**Identification (1)**

Determine the absorption spectrum of a solution of Pyrrolnitrin in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry \( \text{<2.45>} \), and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pyrrolnitrin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pyrrolnitrin as directed in the potassium bromide disk method under Infrared Spectrophotometry \( \text{<2.25>} \), and compare the spectrum with the Reference Spectrum or the spectrum of Pyrrolnitrin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** \( \text{<2.60> 124 – 128°C} \)

**Purity**

Related substances—Dissolve 0.10 g of Pyrrolnitrin in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 3 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography \( \text{<2.03>} \). Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of xylene, ethyl acetate and formic acid (18:2:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly diluted sulfuric acid (1 in 3) on the plate, and heat at 100°C for 30 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**Loss on drying** \( \text{<2.41> Not more than 0.5% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours)} \)

**Residue on ignition** \( \text{<2.44> Not more than 0.1% (1 g)} \)

**Assay**

Conduct this procedure using light-resistant vessels. Weigh accurately an amount of Pyrrolnitrin and Pyrrolnitrin RS, equivalent to about 50 mg (potency) each, and dissolve separately in diluted acetonitrile (3 in 5) to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution, add diluted acetonitrile (3 in 5) to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography \( \text{<2.01>} \) according to the following conditions, and calculate the ratios, \( Q \), and \( Q_o \), of the peak area of pyrrolnitrin to that of the internal standard.

\[
M_o = \frac{M}{S_o} \times Q \times Q_o \times 1000
\]

**Internal standard solution**—A solution of benzyl benzoate in diluted acetonitrile (3 in 5) (3 in 500).

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of pyrrolnitrin is about 9 minutes.

**System suitability**—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, pyrrolnitrin and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pyrrolnitrin to that of the internal standard is not more than 1.0%.

**Containers and storage**

Containers—Tight containers. Storage—Light-resistant.

**Quinapril Hydrochloride**

キナプリル塩酸塩

\[ \text{C}_{25} \text{H}_{30} \text{N}_2 \text{O}_5 \cdot \text{HCl}: 474.98 \]

(3S)-2-[[2S,2′]-[[1S]-3-carboxylic acid monohydrate]

Quinapril Hydrochloride contains not less than 99.0% and not more than 101.0% of \( \text{C}_{25} \text{H}_{30} \text{N}_2 \text{O}_5 \).